The
Oxoid Manual
of
Culture Media,
Ingredients
and other
Laboratory
Services

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INTRODUCTION

DEHYDRATED CULTURE MEDIA

Dehydrated culture media, which constitute a major part of the Oxoid range, possess many advantages due to their economy, ease of storage and preparation, and most of all to the absolute constancy and tested reliability which can only be obtained by large-scale production allied to extensive quality control. For these reasons Oxoid media have become universally accepted and are especially recommended for use in standard methods or other purposes where standardization and reproducibility are of paramount importance. In addition, the ease of preparation of Oxoid dehydrated culture media has enabled laboratories to deal with an ever-increasing amount of work whilst actually reducing the number of staff engaged in the routine tasks of media production.

TABLETS AND POWDERS

A special feature of Oxoid dehydrated culture media is that many of them are available in two different physical forms—tablets or powders.

Tablets are a unique feature of the Oxoid range: each tablet contains a measured amount of mixed ingredients sufficient to make up 5 or 10 ml of medium. All that is necessary is to dissolve the tablet in distilled water and then to sterilize. Tablets are the most convenient form of dehydrated culture media for the preparation of small quantities of media in flasks or in test-tubes. Those who have suffered the labours involved in dispensing a hot agar medium will appreciate the convenience of merely taking a tablet, dropping it into the final container with the correct volume of distilled water, and sterilizing directly in the autoclave. In the case of Cooked Meat Medium, Liver Broth and other media with insoluble constituents, tube-to-tube uniformity is far easier to achieve by using tablets instead of preparing the medium in bulk and then distributing it into smaller containers.

The granule form of dehydrated culture media is still maintained for some products such as Cooked Meat Medium and Liver Broth.

QUALITY CONTROL

The quality control of Oxoid culture media is based upon three concepts:

1. Control of the purity of raw materials.
2. Control of the process.
3. Examination of the final product.

The methods employed are designed to ensure satisfactory microbiological performances by the product, and at the same time to provide consistency between batches. No material is dispatched from Oxoid without first having been subjected to a laboratory examination governed by rigid specifications; complete records are kept for reference.

1. Control of the Purity of Raw Materials

(i) Chemicals. These are either of Analytical Reagent quality or conform to Pharmacopoeia standards. Bulk buying ensures consistency over long periods of manufacture, and sample analysis before purchase guarantees suitability for inclusion in Oxoid Culture Media.

(ii) Dyes, Carbohydrates and Reagents. These are of guaranteed purity and uniformity, prepared to Oxoid specifications and exhaustively tested in our own laboratories before use.

(iii) Raw Materials of Animal and Plant Origin. Oxoid is its own manufacturer of peptones, bile salts, hydrolysates, infusions and extracts. Years of experience in this specialized field have enabled us to produce culture media ingredients of constant composition and finest quality. These ingredients form the basis of Oxoid Culture Media. These media and the ingredients from which they are prepared must conform, where required, to formulae and standards laid down in various official publications including those of the American Public Health Association (1958, 1963, 1965, 1966, 1967), Cruickshank (1965), Society for General Microbiology (1956), Stokes (1968), Windle Taylor (1958), and the British and United States Pharmacopoeias. In particular, inhibitory agents such as bile salts are checked bacteriologically and incorporated in culture media in sufficient quantity to give no more and no less than a defined level of inhibition.

2. Control of the Process

Very close attention is paid to all stages of the manufacture of Oxoid Culture Media products. Samples are withdrawn at intermediate stages and submitted to laboratory tests for growth efficiency and microbiological purity. Continuous recording apparatus and stringent 'in-process' controls over a production line of modern specialized equipment, contribute towards the production of culture media in their most up-to-date and progressive form. The manufacture of Oxoid Media as tablets and powders represents a distinct advance in the presentation of this type of product, and is based upon processes developed within the organization. The processes are carried out using modern equipment of stainless steel and glass, so that at no time is the product contaminated with toxic metals. Plant hygiene is controlled by the routine bacteriological examination of rinse waters and equipment.

3. Examination of the Final Product

Before the release of a batch of Oxoid Dehydrated Medium the final product must pass all the tests imposed upon it by the Quality Control Laboratory. These tests are designed to ascertain the colour, clarity, pH, gel strength (where applicable), moisture, etc., so that no physical variations disturb the batch to batch uniformity of the product. Large scale manufacture to meet world-wide demand contributes towards standardization and eliminates the variables associated with the Laboratory preparation of culture media.

The bacteriological control of every batch of medium, whether in powders or tablet form, is influenced by the intended use of the product, and the choice of test organisms employed is governed by this factor. In all the tests imposed—be they of growth promotion, differentiation, biochemical performance or selectivity—use is made of inocula containing small numbers of organisms (5 to 20). By this means it is possible to ascertain the performance of the medium under exacting conditions, unaided by massive inoculation or masked by the transfer of extraneous growth substrate. The drop technique of Miles and Misra (1938), described by E. Joan Stokes (1968) in her chapter on media testing, forms a valuable part of the quality control procedures on Oxoid products.

Clinical trials carried out in conjunction with hospital, university and industrial laboratories enable the product to be examined under working conditions with a wide range of organisms, over extended periods.

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GENERAL GUIDE TO THE USE OF OXOID PRODUCTS

RECONSTITUTION AND STERILIZATION

The undermentioned instructions apply to the majority of dehydrated culture media, but where there are exceptions to the general rule they will be dealt with individually at the end of this section.

Stage 1. Getting the Product Into Solution

(a) Media not containing agar. When broth media dissolve in distilled water they form a dense layer of concentrated broth syrup on the bottom of the flask or container. This heavy syrup tends to remain at the bottom, where, when heat is applied, destruction may occur through superheating, hydrolysis, caramelization, pH drift, etc. Therefore, thorough mixing is necessary before heat is applied.

(b) Media containing agar. (i) Every agar preparation must be suspended in distilled water and dissolved, by bringing the suspension to the boil, before it is autoclaved.

(ii) In order to dissolve an agar medium it must be boiled during reconstitution. Agar is insoluble in water below 98–100° C, therefore, if a medium is to be prepared in bulk, prior to distribution in smaller units, e.g. bottles or flasks, it must be brought to the boil in order to secure solution and uniform distribution of the agar. Failure to do so will result in the undissolved agar settling to the bottom of the bulk container and being unevenly distributed into final smaller units. An agar medium may be dissolved by 'flash' autoclaving (i.e. raising the pressure momentarily to attain a temperature of 108–115° C), by boiling on a gauze over a flame, or (for smaller units) by holding in free flowing steam for 30 minutes. The latter method is particularly recommended for re-melting agar gels.

If the agar is to be dissolved by boiling, agitate continuously to avoid charring of the medium. The base of the flask is very hot and the agar granules will stick and burn if they are allowed to settle on the bottom. Use a container with a capacity approximately 2½ times the volume of the medium, to allow plenty of room for mixing and frothing.

When the mixture just begins to boil, remove the source of heat and stand the flask on the bench until the frothing has subsided. Quickly return the flask to the hot gauze (without the flame) and allow the medium to simmer for 1 to 2 minutes. Remove the flask once again if frothing becomes too violent. Complete solution of the agar is indicated when the subsiding froth leaves the sides of the flask clear of agar 'tears'.

Cool to 50–60° C before distribution into final containers. Boiling agar is dangerous to handle, may crack cool glass, and gives rise to excessive condensation. Mix well before pouring—insoluble components which, in some cases, are an integral part of the formulation, must be thoroughly resuspended during distribution.

(c) Tablets not containing agar. Oxoid broth tablets are selfmixing. Simply put a tablet into 10 ml of distilled water, in a test-tube or bottle, and heat sterilize. After sterilization, a uniform broth solution will be available ready for inoculation at a lower temperature. Do not use a test-tube or container less than 16 mm (⅝ in.) internal diameter for quantities up to 10 ml; for 20 to 40 ml use a container with a diameter of not less than 25 mm (1 in.); for larger volumes employ a flask or bottle. Narrow tubes or restricted bottles interfere with the 'self-mixing' action of the tablets.

(d) Tablets containing agar. All tablets containing agar must be allowed to soak in cold distilled water for 5 minutes. As the tablets soak in cold water they disintegrate and on heating become self-mixing—hence it is not necessary to mix mechanically. Tablets may be dissolved by autoclaving or by boiling. If by boiling, merely place the tube containing the disintegrated tablet in a cold water bath so that the contents of the tube are below the water line. Bring the bath to the boil and continue boiling until the agar 'boils' in its tube. Complete solution can be judged by the absence of agar 'tears' from the sides of the glass.

Oxoid tablets, for the preparation of agar media, usually require the addition of 5 ml of water during reconstitution. For the preparation of plates, use 2 or 3 tablets and 10 or 15 ml of water; for 'stabs' 2 tablets and 10 ml of water; for slopes, 1 tablet and 5 ml of water.

(e) Special cases. (i) Diagnostic Sensitivity Test Agar CM261 and other media containing dextrose and a phosphate buffer may darken if superheated as a concentrated solution. To avoid this it is best to add the granules to water which has been warmed to 60° C. This procedure assists rapid solution and uniform distribution of the carbohydrate and buffer, thus lessening the possibility of concentrated syrup settling at the bottom of the container.

(ii) Kohn Two-Tube Medium No. 2 CM181, Thioglycollate Medium (Brewer) CM23, Thioglycollate Medium USP CM173 and Stuart Transport Medium CM111 all contain low concentrations of agar. If the agar is not completely dissolved prior to distribution there is a risk of obtaining some tubes of media which contain either too much or too little agar. Therefore it is necessary to boil to dissolve completely before tubing and autoclaving. The weak gels formed by these media are liable to separate due to localized cooling if cooled too rapidly after sterilization—e.g. by placing the hot tubes on a cold surface. For this reason the hot distributed medium should preferebly be allowed to cool slowly on a draught-free non-conducting surface.

(iii) Cooked Meat Medium CM82 and Liver Broth CM78 tablets should be soaked for 5 minutes. These tablets, when added to water without adequate soaking, are subject to rapid pressure changes during autoclaving: they may disintegrate violently due to the release of trapped air, and vigorous frothing may carry meat particles up to the neck of the container. Therefore it is recommended that Liver Broth and Cooked Meat Medium

tablets should be steamed in their final container, prior to sterilization, to remove trapped air.

Stage 2. Sterilization

(i) Sterilize by autoclaving or boiling according to the directions for individual preparations. The times and temperatures recommended relate to the temperatures achieved by the medium and the length of time held at the specified temperature. It should be appreciated that only small quantities of media (i.e. less than 100 ml) heat up at the same rate as the average autoclave. Allowance should be made for a suitable heat-penetration time to be added to the recommended sterilization time, when larger volumes are autoclaved.

Individual autoclaves vary in their heat transferability and should therefore be checked by placing thermo-couples in the media containers. However, the following time intervals for heat penetration of media in glass bottles, at 121° C, are offered for guidance:

250 ml bottle	12 minutes
500 ml bottle	18 minutes
1,000 ml bottle	22 minutes
2,000 ml bottle	27 minutes
5,000 ml bottle	37 minutes

These time intervals may be added to the recommended 15 minutes to ensure that the volume of medium is at 121° C for the prescribed time.

Since culture media deteriorate on over-heating, it follows that the sterilization of media in large bulk volumes should be avoided, unless the heat transfer can be made very efficient, e.g. a plate heat-exchange sterilizer.

(ii) The capacity of containers should be sufficient to provide a generous head space to allow for frothing.

(iii) Screw cap closures must be a half turn free to allow flow of 'tidal' air during heat treatment. These closures are screwed down tightly only after sterilization is complete.

(iv) Containers should be alkali-free to prevent alteration of the pH of the contents. They should not be removed from the sterilizer while still hot, as a sudden change in internal vapour pressure in the containers will give rise to explosive ebullitions. Cool to 70–80° C before opening the sterilizer.

Stage 3. Additions to the Medium After Sterilization

Where the directions call for the addition of a sterile reagent to the medium after autoclaving, this must be carried out with aseptic precautions, taking care to observe the conditions of temperature prescribed.

If the addition consists of a significant amount of enrichment or fluid inoculum then the amount of such an addition should be subtracted from the volume of distilled water required for reconstitution of the medium. In this way the concentration of the ingredients in the formula will not be lowered and the gel strength of agar preparations will not be weakened.

Blood for the preparation of blood agar should be as fresh as possible and should not have been stored below 4° C (e.g. too near the freezing compartment). Temperature fluctuations should be avoided, e.g. the blood should not repeatedly be allowed to warm up to room temperature and then returned to the refrigerator. To avoid thermal shock it is advisable to warm the blood in a 37° C incubator before addition to the sterile molten agar base which has been cooled to 45–50° C. The volume of the container used for mixing blood agar should be approximately 2½ times the total volume of the medium to ensure adequate aeration of the blood. Poorly oxygenated blood plates are purplish whilst adequately aerated plates are a bright cherry red.

Stage 4. Remelting

Most agar gels will withstand one remelting following sterilization and gelling, an advantage where it is desired to store bottled media in a sterile ready to use form. However, it must be emphasized that media which have been remelted are prone to precipitation if held in a molten state for more than 2–3 hours—particularly at temperatures ranging from 40° C to 50° C. Such media may also undergo nutritional impairment if held in the molten state for too long.

SOME RESULTS OF INCORRECT RECONSTITUTION

Making up dehydrated media is simple and easy to carry out, nevertheless, difficulties do occasionally arise—and they can usually be traced to one or more of the causes set out below:

Fault	*Possible Causes*
1. Drift in pH	Super-heating, incomplete mixing; excessive sterilization; use of alkaline glass; contaminated containers; impure distilled water; repeated re-melting, or prolonged storage at high temperature; hydrolysis of ingredients; pH determination carried out on hot medium—allow to cool to room temperature for 2 hours before checking the pH.
2. Incomplete solubility	Inadequate soaking; inadequate heating; incomplete mixing, causing super-heating; use of tap water; container too small to allow adequate convection. (Note: A precipitate may be an essential part of the medium, e.g. Eosin Methylene Blue Agar, Bismuth Sulphite Agar.)
3. Darkening	Super-heating due to incomplete mixing.
4. Soft Gel	Agar not in solution; incomplete mixing; incorrect proportions of dehydrated medium to volume of water; pH drift due to acid hydrolysis ('acid' agars, e.g. Malt Extract Agar, will hydrolyze on prolonged heating or if repeatedly re-melted); failure to compensate for dilution of agar by the inoculum.
5. Loss of growth promotion or differential properties	Repeated re-melting; prolonged or excessive heating; incomplete mixing; charring or burning; failure to compensate for dilution of ingredients by inoculum; disturbance of the formula by the inoculum, e.g. the addition of strong electrolytes or sugar solution, detergents, antiseptics, metallic poisons, protein material, etc.

STORAGE OF OXOID PRODUCTS

Dehydrated Media (CM, MM Series) and Laboratory Products (L Series)

Tablets and powders are supplied in specially sealed screw-capped bottles which should be kept at an even temperature in a cool, dry place, away from direct light. All dehydrated media are hygroscopic and once the bottle has been opened the remaining contents should be protected from atmospheric moisture by replacing the inner seal and the screw-cap immediately after use. It is particularly important not to leave bottles open in the humid atmosphere close to autoclaves and boiling water baths, etc., or for long periods during weighing operations.

Reconstituted Media

The useful life of a culture medium, once prepared, depends on a number of factors, and whilst for the most critical work it is desirable to use them on the day of preparation, many media may safely be stored in a refrigerator for several weeks before use. Obviously, liquid or solid media in screw-cap containers are more suitable for prolonged storage than media in plates, or in containers plugged with cotton-wool.

Moderate refrigeration at approximately + 4° C prolongs the shelf-life of many media, a notable exception being thioglycollate media, which should be stored at room temperature in the dark. After storage, it is advisable to incubate several 'controls' in order to check sterility. Contamination with condensation water may be minimised by not pouring solid media until they have cooled sufficiently and by allowing excess moisture to evaporate before refrigeration. Poured plates are best stored inverted. Media which have been stored in a refrigerator should be allowed to warm up gradually to the incubation temperature before inoculation—this avoids the delayed initiation of growth due to a cold medium and also allows 'misted' containers to clear.

Ready Prepared Media (PM and R Series)

Oxoid Ready Prepared Media are supplied, sterilized, in screw-capped bottles. Store at 4° C where indicated, or in a cool dark place. Humidity conditions are not important.

Biochemical and Sterile Reagents (BR and SR Series)

Biochemical Reagents and Sterile Reagents should be protected from high temperatures, light and microbial contamination. As a general guide, those products in liquid form, e.g. Horse Blood, Serum, ampoules of reagents, should be stored at 4° C. Calf serum however should be stored frozen. Those products in dry form should be stored in a cool, dark place and protected from moisture.

For more details please consult the section of the Manual dealing with the particular product.

SPECIFIC GUIDES TO THE USE OF OXOID PRODUCTS

OXOID PRODUCTS FOR SPECIFIC GROUPS OF MICRO-ORGANISMS

NB Blood Agar Base Media (see list in Manual)

Organism	*Isolation/Cultivation*	*Identification*	*Maintenance*
Actinomycetes (aerobic): *Nocardia* *Streptomyces*	Brain Heart Infusion Czapek Dox Agar Sabouraud Dextrose Agar Sabouraud Maltose Agar Tryptone Soya Agar Tryptone Soya Broth	Loeffler Serum Medium Nutrient Gelatin	Tryptone Soya Agar
Anaerobes, including *A. bovis* *Bacteroides* *Clostridium*	Blood Agar Base Media Brain Heart Infusion Clausen Medium Cooked Meat Medium (Synthetic) Iron Sulphite Agar Liver Broth Nutrient Broth Reinforced Clostridial Medium Reinforced Clostridial Agar Schaedler Medium Thioglycollate Medium (Brewer) Thioglycollate Medium (USP) Tryptone Soya Agar Tryptone Soya Broth	Charcoal Gelatin Discs Concentrated Egg York Emulsion Cooked Meat Medium (Synthetic) Crossley Milk Medium Fildes Extract Iron Sulphite Agar Loeffler Serum Medium Peptone Water Sugars Tryptone Water	Cooked Meat Medium (Synthetic) Liver Broth
Bacillus	Dextrose Tryptone Agar Nutrient Agar Nutrient Broth Shapton Medium Tryptone Soya Agar Tryptone Soya Broth	Charcoal Gelatin Discs Concentrated Egg Yolk Emulsion Crossley Milk Medium Peptone Water Sugars	Nutrient Broth Tryptone Soya Agar Tryptone Soya Broth
Bordetella	Bordet-Gengou Agar 'Chocolate' Agar (CM271) Charcoal Blood Agar	Peptone Water Sugars X + V factor discs on Blood Agar Base (CM55)	
Brucella	Brucella Medium Liver Broth Tryptone Soya Agar Tryptone Soya Broth	Brucella Medium with fuchsin with thionin	Brucella Medium Columbia Blood Agar Base
Coliforms	**RESUSCITATION MEDIA** EE Broth Membrane Enriched Teepol Broth Resuscitation Membrane Broth **ISOLATION MEDIA** Blood Agar Brilliant Green Bile Broth China Blue Lactose Agar CLED Medium Cooked Meat Medium Desoxycholate Agar Endo Agar Base Eosin Methelene Blue Agar Hektoen Enteric Agar Lactose Broth Lauryl Tryptose Broth MacConkey Agar MacConkey Broth MacConkey Membrane Broth Minerals Modified Glutamate Medium Nutrient Agar Nutrient Broth Plate Count Agar Purple Serum Agar Tryptone Soya Agar Tryptone Soya Broth Violet Red Bile Agar XLD Medium	Brilliant Green Bile Broth Koser Citrate Medium Lactose Ricinoleate Broth Lauryl Tryptose Broth MRVP Medium Nutrient Gelatin Peptone Water Sugars SIM Medium Simmons Citrate Agar Tryptone Water	Nutrient Agar Tryptone Soya Agar

Organism	Isolation/Cultivation	Identification	Maintenance
Corynebacterium	Columbia Blood Agar Base Hoyle Medium Base Loeffler Serum Medium Nutrient Broth Tryptone Soya Agar Tryptone Soya Broth	Blood Agar Charcoal Gelatin Discs Hoyle Medium Base Loeffler Serum Medium Nutrient Gelatin	Loeffler Serum Medium Tryptone Soya Agar
Gram-positive Cocci: *Diplococcus* } *Streptococcus* }	Azide Blood Agar Blood Agar Base Media Brain Heart Infusion China Blue Lactose Agar Dextrose Broth Dextrose Peptone Agar Dextrose Peptone Broth D.S.T. Blood Agar Edwards Medium Mitis Salivarius Agar Nutrient Broth Purple Serum Agar Base Todd-Hewitt Broth Tryptone Soya Agar Tryptone Soya Broth	Azide Blood Agar Bacitracin Discs Blood Agar Base Media Cooked Meat Medium (Synthetic) Edwards Medium Mitis Salivarius Agar Optochin Discs Peptone Water Sugars	Blood Agar Base Media Cooked Meat Medium (Synthetic) Stuart Transport Medium Tryptone Soya Agar
Enterococcus	Blood Agar Base Media China Blue Lactose Agar MacConkey Agar No. 2 MacConkey Broth Purple Serum Agar Slanetz and Bartley Medium	Azide Blood Agar Blood Agar Nutrient Gelatin Purple Serum Agar	Blood Agar Base Media Cooked Meat Medium (Synthetic) Tryptone Soya Agar
Staphylococcus	Baird-Parker Medium Blood Agar Base Media China Blue Lactose Agar KRANEP Agar MacConkey Agar Mannitol Salt Agar Nutrient Broth Salt Meat Broth Staphylococcus Medium No. 110 Tryptone Soya Agar Tryptone Soya Broth	Baird-Parker Medium Blood Agar Base Media Charcoal Gelatin Discs Egg Yolk Emulsion DNase Agar Egg Yolk Tellurite Emulsion Loeffler Serum Medium Mannitol Salt Agar Nutrient Gelatin Phenolphthalein Phosphate Agar Staphylococcus Medium No. 110	Blood Agar Base Media Cooked Meat Medium (Synthetic) Tryptone Soya Agar
Haemophilus	Blood Agar Base Media Charcoal Blood Agar Fildes Agar	Charcoal Agar V Factor Discs X Factor Discs X + V Factor Discs	Charcoal Agar 'Chocolate Agar' Tryptone Soya Agar
Lactobacillus and *Leuconostic*	Milk Agar M.R.S. Agar Plate Count Agar Reinforced Clostridial Agar Reinforced Clostridial Medium Rogosa Agar Schaedler Medium Skim Milk Powder Tomato Juice Agar Tryptone Soya Agar Tryptone Soya Broth	M.R.S. Broth Peptone Water Sugars Tomato Juice Agar	Liver Broth Tryptone Soya Broth
Listeria	Brain Heart Infusion Tryptone Soya Agar Tryptose Blood Agar	MRVP Medium Peptone Water Sugars	Tryptone Soya Agar
Mycobacterium	Acid Egg Medium Bordet-Gengou Agar Buffered Pancreatin Tablets Dorset Egg Medium Lowenstein-Jensen Medium Pyruvic Acid Egg Medium	Lowenstein-Jensen Medium	Dorset Egg Medium Lowenstein-Jensen Medium

Organism	Isolation/Cultivation	Identification	Maintenance
Mycoplasma	Blood Agar Base No. 2 Columbia Blood Agar Base Mycoplasma Agar and supplements Mycoplasma Broth and supplements	Mycoplasma Media and supplements	Mycoplasma Media and supplements
Neisseria	Blood Agar Base Media Charcoal Blood Agar 'Chocolate Agar' GC Agar and supplements Mueller Hinton Agar Mueller Hinton Broth Tryptone Soya Agar Tryptone Soya Broth	Loeffler Serum Medium	Stuart Transport Medium
Pasteurella	Blood Agar Base Media MacConkey Agar Tryptone Soya Agar Tryptone Soya Broth	Blood Agar Base Media MacConkey Agar Peptone Water Sugars Urea Agar Urea Broth	Blood Agar Base Media Tryptone Soya Broth
Proteus	Blood Agar Base Media CLED Medium Cooked Meat Medium (Synthetic) E.E. Broth Lab Lemco Agar MacConkey Agar Nutrient Agar Nutrient Broth Tryptone Soya Agar Tryptone Soya Broth	Charcoal Gelatin Discs Kohn Two-Tube Medium Koser Citrate Medium Peptone Water Sugars Phenylalanine Agar Simmons Citrate Agar Tryptone Water Triple Sugar Iron Agar	Blood Agar Base Media Cooked Meat Medium (Synthetic) Nutrient Agar Tryptone Soya Agar Tryptone Soya Broth
Pseudomonas	Brain Heart Infusion Cooked Meat Medium (Synthetic) Nutrient Agar Tryptone Soya Agar		Cooked Meat Medium (Synthetic)
Salmonella and *Shigella*	**ENRICHMENT MEDIA** Mannitol Selenite Broth Base Muller-Kauffman Tetra-thionate Broth Selenite Broth Base Tetrathionate Broth Base **ISOLATION MEDIA** Bismuth Sulphite Agar Brain Heart Infusion Brilliant Green Agar Brilliant Green Agar (modified) Cooked Meat Medium (Synthetic) DCLS Agar Desoxycholate Citrate Agar Desoxycholate Citrate Agar (Hynes) EE Broth Endo Agar Base Hektoen Enteric Agar MacConkey Agar MacConkey Membrane Broth Nutrient Agar Nutrient Broth SS Agar Tryptone Soya Agar Tryptone Soya Broth Violet Red Bile Dextrose Agar XLD Medium	Hektoen Enteric Agar Kliger Iron Agar Kohn Two-Tube Medium Lysine Decarboxylase Broth Lysine Iron Agar MRVP Medium Peptone Water Sugars Simmons Citrate Agar Triple Sugar Iron Agar Tryptone Water Urea Agar Urea Broth	Blood Agar Base Media Cooked Meat Medium (Synthetic) Nutrient Agar Nutrient Broth Tryptone Soya Agar Tryptone Soya Broth

Organism	*Isolation/Cultivation*	*Identification*	*Maintenance*
Vibrio	Cholera Medium DCLS Peptone Water TCBS Cholera Medium Tryptone Soya Agar Tryptone Soya Broth	Charcoal Gelatin Discs Cholera Medium Koser Citrate Medium MRVP Medium Nutrient Gelatin Peptone Water Simmons Citrate Medium TCBS Cholera Medium Tryptone Water	Cooked Meat Medium (Synthetic)
Yeasts and Moulds	'Actidone' Agar Brain Heart Infusion Buffered Yeast Agar Corn Meal Agar Czapek Dox Agar Eosin Methylene Blue Agar Lysine Medium Malt Extract Agar Malt Extract Broth Malt Extract Membrane Broth Potato Dextrose Agar Sabouraud Medium Sugar Free Agar Trichomonas Medium Tryptone Soya Agar Tryptone Soya Broth WL Nutrient Medium Wort Agar	Corn Meal Agar Czapek Dox Agar Eosin Methylene Blue Agar Lysine Medium Peptone Water Sugars Sabouraud Medium Trichomonas Medium WL Nutrient Medium	Corn Meal Agar Malt Extract Agar Sabouraud Medium Tryptone Soya Agar Wort Agar

A GUIDE TO RECOMMENDED CULTURE MEDIA

This table is a guide to the specimens usually examined in microbiological laboratories. The culture media listed are those which are recommended for the examination of such specimens. This list is not intended to be exhaustive **AND DOES NOT NECESSARILY READ ACROSS.** More information may be obtained from the table of Oxoid products (pp. 20-26) on specific groups of micro-organisms and from the alphabetical section in this Manual.

Specimen and Micro-Organisms	*Disease or Infection*	*Culture Media*
BLOOD CULTURE		
Anaerobic pathogens e.g.		Blood Agar Base No. 2
Bacteroides		Brain Heart Infusion
Clostridium	gangrene	Brain Heart Infusion Agar
Brucella	brucellosis (undulant fever)	Brucella Medium Base
gram-negative cocci e.g.		Chocolate Agar
Neisseria	acute septicaemia	Clausen Medium
gram-positive cocci e.g.		Columbia Blood Agar Base
		Cooked Meat Medium (Synthetic)
Diplococcus		DNase Agar
Staphylococcus		Fildes Extract
Streptococcus	acute septicaemia	GC Agar Base and supplements
gram-negative rods e.g.		Hartley Digest Broth
Haemophilus		Mycoplasma Agar Base and supplements
Pasteurella	plague	Reinforced Clostridial Medium
Pseudomonas		Sabouraud Dextrose Agar
Salmonella	enteric fever	Thioglycollate Medium U.S.P.
		Tryptone Soya Agar
gram-positive rods e.g.		Tryptone Soya Broth
Bacillus	anthrax	Tryptose Blood Agar Base
Listeria	listeriosis	Tryptose Phosphate Broth
Histoplasma		
Mycoplasma (PPLO)		
pathogenic fungi	systemic mycoses	
GENITO-URINARY SPECIMENS		
Anaerobic pathogens e.g.		Blood Agar Base
Bacteroides		Blood Agar Base No. 2
Clostridium	septic abortion	Brain Heart Infusion Agar
Candida albicans	vaginitis	Charcoal Agar
gram-negative cocci e.g.		C.L.E.D. Medium
Neisseria	gonorrhoea	C.L.E.D. (with Andrade indicator)
gram-positive cocci e.g.		Columbia Blood Agar Base
Staphylococci		Corn Meal Agar
Streptococci	puerperal fever	Czapek Dox Agar
gram-negative rods e.g.		Eosin Methylene Blue Agar
Coliforms	urogenital tract infections	G.C. Agar Base and supplements
Haemophilus	chancroid	MacConkey Agar No. 2
Proteus		Purple Serum Agar Base
Pseudomonas		Stuart Transport Medium
Trichomonas	trichomoniasis	Trichomonas Medium
		Trichomonas Medium No. 2
		Tryptose Blood Agar Base
		Urea Agar Base
		Urea Broth Base

Specimen and Micro-Organisms	*Disease or Infection*	*Culture Media*
EAR CULTURES		
gram-positive cocci e.g.		Blood Agar Base No. 2
Diplococcus	otitis media	Charcoal Agar
Staphylococcus		Chocolate Agar
gram-negative rods e.g.		Columbia Blood Agar Base
Coliforms		Desoxycholate Agar
Haemophilus		DNase Agar
Proteus	secondary infections	Hoyle Medium Base
Pseudomonas		Loeffler Serum Medium
gram-positive rods e.g.		MacConkey Agar
Corynebacterium		Mannitol Salt Agar
pathogenic fungi		Nutrient Broth No. 2
		Sabouraud Dextrose Agar
		Tryptone Soya Broth
		Tryptose Phosphate Broth
EYE CULTURES		
Candida		Blood Agar Base No. 2
gram-negative cocci e.g.		Chocolate Agar
Neisseria	opthalmia	Columbia Blood Agar Base
		Corn Meal Agar
gram-positive cocci e.g.		Czapek Dox Agar
Diplococcus		Desoxycholate Agar
Staphylococcus	styes, blepharitis	Eosin Methylene Blue Agar
Streptococcus		G.C. Agar Base and supplements
gram-negative rods e.g.		Hoyle Medium Base
Haemophilus	conjunctivitis	Loeffler Serum Medium
Klebsiella		Purple Serum Agar
Proteus		Sabouraud Dextrose Agar
Pseudomonas		Tryptose Blood Agar Base
gram-positive rods e.g.		Tryptose Phosphate Broth
Corynebacterium		
ENTERIC PATHOLOGICAL SPECIMENS		
anaerobic pathogens e.g.		Azide Blood Agar Base
		Bismuth Sulphite Agar
Clostridium	food poisoning	Blood Agar Base
Candida	monilial enteritis	Brilliant Green Agar

Specimen and Micro-Organisms	*Disease or Infection*	*Culture Media*
gram-negative rods e.g.		Cholera Medium
Esch. Coli	gastro-enteritis	Cooked Meat Medium (Synthetic)
Proteus		Desoxycholate Citrate Agar
		D.C.L.S. Agar
Pseudomonas		Eosin Methylene Blue Agar
Salmonella	salmonellosis { enteric fever	Hektoen Enteric Agar
	typhoid fever	MacConkey Agar
Shigella	bacillary dysentery	MacConkey Agar No. 2
gram-positive cocci e.g.		MacConkey Agar No. 3
Staphylococcus	food poisoning	Reinforced Clostridial Agar
Streptococcus		Schaedler Medium
Vibrio	cholera	Selenite Broth Base
		SS Agar
		Staphylococcus Medium No. 110
		TCBS Medium
		Tetrathionate Broth Base
		Tryptose Blood Agar Base
		Urea Agar Base
		Urea Broth Base
		XLD Medium
SKIN, HAIR AND NAIL CULTURES		
pathogenic fungi e.g.		Blood Agar Base No. 2
Candida	moniliasis	Corn Meal Agar
Epidermophyton	skin, nail infection	MacConkey Agar
Microsporum	epidemic ringworm,	Sabouraud Agar
	tinea capitis	Staphylococcus Medium No. 110
Trichophyton	ringworm	Tryptose Blood Agar Base
Pseudomonas		
Staphylococcus	boils	
Streptococcus	impetigo	
SPINAL AND OTHER FLUIDS		
Bacteroides		Blood Agar Base No. 2
Cryptococcus	systemic infection	Brain Heart Infusion Agar
Diplococcus		Chocolate Agar
Haemophilus	meningitis	Dorset Egg Medium
Mycobacterium	tuberculous meningitis	G.C. Agar Base and supplements
Neisseria	meningitis	Lowenstein-Jensen Medium
Nocardia	nocardiosis	Sabouraud Agar
Streptococcus		Tryptose Blood Agar Base
THROAT AND NOSE SPECIMENS		
Candida	thrush	Blood Agar Base No. 2
gram-negative cocci e.g.		Bordet-Gengou Agar Base
Neisseria	meningitis	Charcoal Agar
gram-positive cocci e.g.		Corn Meal Agar
Diplococcus	pneumonia	Czapek Dox Agar
		Eosin Methylene Blue Agar
Streptococcus	septic sore throat,	Hoyle Medium Base
	scarlet fever	Loeffler Serum Medium

Specimen and Micro-Organisms	Disease or Infection	Culture Media
gram-negative rods e.g.		Sabouraud Agar
Bordetella	whooping cough	Todd-Hewitt Broth
Haemophilus	sinusitis	Tryptone Soya Agar
Klebsiella		
gram-positive rods e.g.		
Corynebacterium	diphtheria	
Listeria		
SPUTUM SPECIMENS		
Actinomycetes	mycoses	Acid Egg Medium
Candida		Blood Agar Base No. 2
Coccidiodes	coccidiomycosis	Brain Heart Infusion Agar
Diplococcus	pneumonia	Chocolate Agar
Haemophilus	chronic bronchitis	Columbia Blood Agar Base
Mycobacterium	tuberculosis	Corn Meal Agar
Mycoplasma	pneumonia	Czapek Dox Agar
Streptococci	bronchitis	Eosin Methylene Blue Agar
		Lowenstein-Jensen Medium
		Mycoplasma Agar Base and supplements
		Pyruvic Acid Egg Medium
		Sabouraud Dextrose Agar
		Tryptose Blood Agar Base
WOUND SPECIMENS		
anaerobic micro-organisms e.g.		Blood Agar Base No. 2
Bacteroides	deep wound infection	Chocolate Agar
		Clausen Medium
Clostridium	tetanus	Columbia Blood Agar Base
	gangrene	Cooked Meat Medium (Synthetic)
gram-positive cocci e.g.		D.C.L.S. Agar
Staphylococcus	suppuration	Hoyle Medium Base
Streptococcus	cellulitis	Loeffler Serum Medium
gram-negative rods e.g.		MacConkey Agar
Proteus		Reinforced Clostridial Agar
Pseudomonas	secondary infection	Reinforced Clostridial Medium
gram-positive rods e.g.		Staphylococcus Medium No. 110
Corynebacterium		Thioglycollate Medium U.S.P.
		Tryptone Soya Agar
		Tryptone Soya Broth

PHARMACEUTICAL LABORATORY PROCEDURES

Test	*Culture Media*
I. STERILITY TESTING	Blood Agar Base Brain Heart Infusion Clausen Medium *Cooked Meat Medium Cooked Meat Medium (Synthetic) Dextrose Peptone Broth Dextrose Tryptone Agar Dextrose Tryptone Broth *Hartley Digest Broth Liver Broth Malt Extract Broth Membrane Media (MM Series) *Nutrient Broth No. 2 Reinforced Clostridial Medium *Sabouraud Liquid Medium Spore Strips *Thioglycollate Medium (Brewer) *Thioglycollate Medium USP *Tryptone Soya Broth

* suitable for use in the techniques described in the British Pharmacopoeia or in the United States Pharmacopoeia.

2. SENSITIVITY TESTING	Antibiotic Assay Media Bacitracin Discs Dextrose Broth Diagnostic Sensitivity Test Agar 'Iso-Sensitest Agar'/Broth' Mueller Hinton Agar 'Multodisk' Optochin Discs Single Discs Tryptose Phosphate Broth Tryptone Soya Agar

EXAMINATION OF FOOD AND WATER

Organisms	*Culture Media*
CANNED FOODS *Anaerobes* e.g. *Clostridium*	Cooked Meat Medium Cooked Meat Medium (Synthetic) Crossley Milk Medium Iron Sulphite Agar Liver Broth Reinforced Clostridial Medium
Enterobacteriaceae	E.E. Broth MacConkey Agar MacConkey Broth (purple) Violet Red Bile Agar Violet Red Bile Dextrose Agar

Organisms	*Culture Media*
Lactobacilli	M.R.S. Agar Tomato Juice Agar
Thermophiles	Dextrose Tryptone Agar Dextrose Tryptone Broth Shapton Medium
Viable organisms	Nutrient Agar Standard Plate Count Agar (A.P.H.A. Tryptone Soya Agar
Yeasts and Moulds	Malt Extract Agar Potato Dextrose Agar Wort Agar
DAIRY PRODUCTS	
Anaerobes	Iron Sulphite Agar Liver Broth Reinforced Clostridial Agar Reinforced Clostridial Medium
Coliforms	Brilliant Green Bile (2%) Broth Eosin Methylene Blue Agar Desoxycholate Agar Lauryl Tryptose Broth MacConkey Broth (purple) Violet Red Bile Agar
Lactobacilli	MRS Agar MRS Broth Rogosa Agar
Lipolytic organisms	Tributyrin Agar
Staphylococci	Baird-Parker Medium KRANEP Agar Staphylococcus Medium No. 110
Streptococci	Azide Blood Agar Base Edwards Medium (modified) Tryptose Phosphate Broth
Total count of viable organisms	Milk Agar Milk Agar (roll tube) Nutrient Broth No. 2 Plate Count Agar Plate Count Agar (roll tube) Standard Plate Count Agar (A.P.H.A.)
Yeasts and moulds	Buffered Yeast Agar Potato Dextrose Agar

Organisms	*Culture Media*
FROZEN FOODS	
Anaerobes	Iron Sulphite Agar Liver Broth Reinforced Clostridial Medium Schaedler Medium Thioglycollate Medium (USP)
Coliforms	Brilliant Green Bile (2%) Broth Endo Agar Base MacConkey Broth (purple) Violet Red Bile Agar
Salmonella and Shigella	Mannitol Selenite Broth Base Muller-Kauffman Tetrathionate Broth Base Bismuth Sulphite Agar Brilliant Green Agar D.C.L.S. Agar Desoxycholate Citrate Agar Hektoen Enteric Agar
Staphylococci	Baird-Parker Medium DNase Agar KRANEP Agar Base Mannitol Salt Agar
Streptococci (particularly enterococci)	Azide Blood Agar Base MacConkey Agar No. 2 Slanetz and Bartley Medium
Total Viable Count	Nutrient Agar Plate Count Agar Standard Plate Count Agar (A.P.H.A.) Tryptone Soya Agar
Yeasts and Moulds	Malt Extract Agar Potato Dextrose Agar Wort Agar
SUGAR, ALCOHOLIC AND OTHER BEVERAGES	
Anaerobic spore forming organisms e.g. *Cl. nigrificans*	Cooked Meat Medium Cooked Meat Medium (Synthetic) Iron Sulphite Agar Liver Broth
Coliforms	Lactose Broth MacConkey Agar MacConkey Broth (purple)
Lactobacilli	MRS Agar Rogosa Agar Tomato Juice Agar
Thermophilic 'flat-sour' micro-organisms and Total Viable Count	Dextrose Tryptone Agar

Organisms	*Culture Media*
Total contaminating bacteria (in yeast)	'Actidione' Agar
Yeasts and moulds	Lysine Medium ('wild' yeasts) Malt Extract Agar W.L. Nutrient Medium Wort Agar
WATER	
Clostridium welchii	Reinforced Clostridial Medium
Coliforms	Brilliant Green Bile (2%) Broth Endo Agar Base Lactose Ricinoleate Broth Lauryl Tryptose Broth MacConkey Agar MacConkey Broth MacConkey Membrane Broth Tablets Membrane Enriched Teepol Broth Minerals Modified Glutamate Medium Resuscitation Membrane Broth Tablets Violet Red Bile Agar
Faecal streptococci	Azide Blood Agar Base MacConkey Agar No. 2 Slanetz and Bartley Medium
Salmonella and Shigella	Bismuth Sulphite Agar Brilliant Green Agar D.C.L.S. Agar Desoxycholate Citrate Agar (Hynes) Selenite Broth Base Tetrathionate Broth Base XLD Medium

MEDIA FOR DAIRY MICROBIOLOGY

Groups of Micro-Organisms/Test	*Purpose*	*Media*	*Code*
Anaerobes (see also thermophilic anaerobes)	Cultivation and enumeration of anaerobes e.g. Clostridium, Lactobacillus and Streptococcus species. R.C.M. is recommended as the diluent in determination of viable counts of anaerobes.	Reinforced Clostridial Agar Reinforced Clostridial Medium (R.C.M.)	CM.151 CM.149
Coli-aerogenes group of the Enterobacteriaceae	Detection of coliform organisms.	Lactose Broth Lauryl Tryptose Broth MacConkey Broth (Purple) Violet Peptone Bile Lactose Broth	CM.137 CM.451 CM.5a CM.223
	Confirmation of the presence of *Esch. coli* at 44° C	Brilliant Green Bile (2%) Broth	CM.31
	Differentiation between lactose and non-lactose fermenting organisms	China Blue Lactose Agar Endo Agar Base	CM.209 CM.479
	Differentiation and enumeration of coliform bacilli	Desoxycholate Agar Eosin Methylene Blue Agar (Levine) Violet Red Bile Agar	CM.163 CM.69 CM.107
	Differentiation of the coli-aerogenes group: (a) Methyl Red and Voges-Proskauer Tests	MRVP Medium	CM.43
	(b) Citrate Utilisation	Simmons Citrate Medium	CM.155
	(c) Production of Indole	Tryptone Water	CM.87
Gelatin-liquefying organisms	Gelatin liquefaction is used as an aid in the identification of certain micro-organisms e.g. *Streptococcus faecalis var. liquefaciens*, pathogenic staphylococci	Charcoal Gelatin Discs (see also Staphylococcus Medium 110)	BR.10 CM.145

MEDIA FOR DAIRY MICROBIOLOGY—*continued*

Groups of Micro-Organisms/Test	*Purpose*	*Media*	*Code*
Gram-negative rod-shaped organisms	Detection and enumeration of pyschrophilic and mesophilic contaminates in butter and processed dairy products	Sugar Free Agar	CM.417
Lactobacilli	Selective isolation and cultivation of Lactobacilli	MRS Agar MRS Broth Rogosa Agar	CM.361 CM.359 PM.221
Lecithinase-producing organisms	Lecithinase activity is used in the identification of certain organisms, e.g. some Bacillus species	Egg Yolk Emulsion Nutrient Agar	SR.47 CM.3
Lipolytic organisms	Isolation of contaminating lipolytic organisms from milk, butter, cream, etc. Also used for the examination of activity of moulds in mould ripened cheese	Tributyrin Agar	PM.4
Micrococci	Enumeration and differentiation of lactose and non-lactose fermenting organisms including micrococci	China Blue Lactose Agar	CM.209
Staphylococci	Isolation and differentiation of pathogenic staphylococci	{Baird-Parker Medium {Egg Yolk Tellurite Emulsion {Egg Yolk Emulsion {KRANEP Agar Base Staphylococcus Medium No. 110	CM.275 SR.54 CM.441 SR.47 CM.145
Streptococci	Selective isolation of streptococci from dairy products containing a mixed flora. Tryptose Phosphate Broth is used with added azide and agar (A.P.H.A.)	Azide Blood Agar Base Edwards Medium (modified) Tryptose Phosphate Broth	CM.259 CM.27 CM.283
Thermophilic flat-sour organisms	Detection and enumeration of flat-sour organisms (*Bacillus stearothermophilus*) in canned milk products and sugar	Dextrose Tryptone Agar/Broth Tryptone Glucose Extract Agar	CM.75/73 CM.127
Thermophilic Anaerobes	Detection and enumeration of thermophilic anaerobes causing sulphide spoilage e.g. *Clostridium sporogenes*	Iron Sulphite Agar	CM.79
Viable Organisms	General viable count of micro-organisms by pour plate or roll tube method	Milk Agar Milk Agar (Roll Tube) Plate Count Agar Plate Count Agar (Roll Tube) Standard Plate Count Agar (A.P.H.A.) Tryptone Soya Agar	CM.21 CM.21a CM.325 CM.325a CM.463 CM.131/132
	General Cultivation	Nutrient Broth No. 2	CM.67
Yeasts and Moulds	Control of bottle washing operations	Buffered Yeast Agar	CM.153
	Enumeration of moulds and yeasts in butter, cream, etc.	Potato Dextrose Agar	CM.139
Plant Hygiene	Diluent or rinse in bacteriological examination of dairy products, plant and apparatus	Ringer Solution Tablets	BR.52
	Solvent diluent solution for calcium alginate swabs	'Calgon' Ringer Tablets	BR.49
	Chlorine-neutralizing Ringer Solution to counteract the bactericidal effect of hypochlorites or other chlorine sources	Thiosulphate Ringer Tablets	BR.48
A.I.M. Test	Determination of penicillin and other inhibitory substances in milk	A.I.M. Test	MT.3

For details of Membrane Filtration Techniques for dairy products, see the separate Oxoid Booklet

MEDIA FOR FOOD MICROBIOLOGY

Groups of Micro-Organisms/Test	Purpose	Media	Code
Anaerobes	Cultivation and enumeration of anaerobic micro-organisms e.g. Clostridia, Lactobacilli and Streptococci. R.C.M. is recommended as the diluent in the determination of viable counts of anaerobes	Cooked Meat Medium (Synthetic) Liver Agar Liver Broth Reinforced Clostridial Agar Reinforced Clostridial Medium (R.C.M.)	CM.439/40 CM.292 CM.77 CM.151 CM.149
	Detection and enumeration of thermophilic anaerobes causing sulphide spoilage	Iron Sulphite Agar	CM.79
	Examination of canned food samples for anaerobic bacteria. Diagnostic tests for the identification of Clostridia species e.g. *Cl. welchii*	Crossley Milk Medium	CM.213
	Confirmation of *Cl. welchii* by Nagler Test	Blood Agar Base Fildes Extract Egg Yolk Emulsion	CM.55 SR.46 SR.47
	Selective isolation of anaerobic organisms from dehydrated or frozen foods	Schaedler Medium	CM.437
Bacillus cereus	Detection of *B. cereus* by lecithinase activity after destroying non spore-forming organisms from foods e.g. rice, pepper and milk	Egg Yolk Emulsion Nutrient Agar	SR.47 CM.3
Coli-aerogenes group	Presumptive coliform tests	Lauryl Tryptose Broth MacConkey Broth (Purple)	CM.451 CM.5a
	Enumeration of coliforms	Violet Red Bile Agar	CM.107
	Confirmation of the presence of *Esch. coli* at 44° C. The Eijkman test in MacConkey Broth may also be used	Brilliant Green Bile (2%) Broth Lactose Ricinoleate Broth MacConkey Broth	CM.31 CM.371 CM.5
	Confirmation of presumptive coliform tests	Endo Agar Base Eosin Methylene Blue Agar	CM.479 CM.69
	Differentiation of the coli-aerogenes group: (a) Methyl Red and Voges-Proskauer tests	MRVP Medium	CM.43
	(b) Citrate utilisation	Simmons Citrate Agar	CM.155
	(c) Production of Indole	Lauryl Tryptose Broth Tryptone Water	CM.451/452 CM.87
Entero-bacteriaceae (see also coli-aerogenes group and Salmonella and Shigella)	Cultivation of Enterobacteriaceae: Resuscitation of sub-lethally impaired cells e.g. in preserved foods, prior to enrichment	Tryptone Soya Broth	CM.129
	Enrichment medium for Enterobacteriaceae in the examination of foods and animal feed stuffs	E.E. Broth	CM.317
	Selective enumeration of Enterobacteriaceae	MacConkey Agar No. 3 MacConkey Agar No. 3 (Roll tube) Violet Red Bile Dextrose Agar	CM.115 CM.115a CM.323
	Differentiation of the Enterobacteriaceae	S.I.M. Medium Triple Sugar Iron Agar Kliger Iron Agar	CM.435 CM.277 CM.33/34
	Detection of urease-producers e.g. *Proteus* group	Urea Agar Base	CM.53
Faecal streptococci (see anaerobes)	Selective enumeration of Lancefield group D Streptococci	Azide Blood Agar Base MacConkey Agar No. 2 Slanetz and Bartley Medium	CM.259 CM.109 CM.377

MEDIA FOR FOOD MICROBIOLOGY—*continued*

Groups of Micro-Organisms/Test	*Purpose*	*Media*	*Code*
Lactobacilli	Selective isolation and enumeration of Lactobacilli e.g. from meats	M.R.S. Agar/Broth	CM.361/359
		Tomato Juice Agar	CM.113
		Rogosa Agar	PM.221
Lipolytic organisms	Cultivation and detection of lipolytic organisms	Tributyrin Agar	PM.4
Salmonella and Shigella	Enrichment procedures prior to isolation	Mannitol Selenite Broth Base	CM.399
		Muller-Kauffman Tetrathionate Broth Base	CM.343
		Selenite Broth Base	CM.395
		Sodium biselenite	L.121
		Tetrathionate Broth Base	CM.29
	Isolation and identification of Salmonella and Shigella species	Bismuth Sulphite Agar	CM.201
		Brilliant Green Agar	CM.263
		Brilliant Green Agar (modified)	CM.329
		D.C.L.S. Agar	CM.393
		Desoxycholate Citrate Agar (Hynes)	CM.227
		Hektoen Enteric Agar	CM.419
		XLD Medium	CM.469
	Identification of Salmonella and Shigella	Kligler Iron Agar	CM.33/34
		Kohn Two-Tube Medium	CM.179/181
		Lysine Iron Agar	CM.381
		Triple Sugar Iron Agar	CM.277/278
Staphylococci	Isolation of coagulase—positive staphylococci	Baird-Parker Medium	CM.275
		Egg Yolk Tellurite Emulsion	SR.54
		Egg Yolk Emulsion	SR.47
		KRANEP Agar Base	CM.441
		Mannitol Salt Agar	CM.85
	Selective enumeration of the total staphylococcal count from foodstuffs	KRANEP Agar Base	CM.441
		Egg Yolk Emulsion	SR.47
	Differentiation of staphylococci:		
	(a) DNase production	DNase Agar	CM.321
	(b) Phosphatase production	Blood Agar Base	CM.55
		Phenolphthalein Phosphate 1%	SR.31
	(c) Coagulase production		
Thermophilic 'flat-sour' micro-organisms	Detection and enumeration of 'flat-sour' organisms in canned goods, sugar, etc.	Dextrose Tryptone Agar	CM.75
		Dextrose Tryptone Broth	CM.73
		Shapton Medium	CM.270
Viable Organisms	General viable count and cultivation of micro-organisms	Blood Agar Base	CM.55
		Nutrient Agar	CM.3
		Nutrient Broth No. 2	CM.67
		Plate Count Agar	CM.325
		Standard Plate Count Agar (A.P.H.A.)	CM.463
		Tryptone Soya Agar	CM.131
		Tryptone Soya Broth	CM.129
Yeasts and Moulds	Cultivation and enumeration of yeasts and moulds	Malt Extract Agar	CM.59
		Potato Dextrose Agar	CM.139
Plant Hygiene and Food Sample Dilution	Diluent or rinse in bacteriological examination of food products, plant and apparatus	Ringer Solution Tablets	BR.52
	Solvent diluent solution for calcium alginate swabs	'Calgon' Ringer Tablets	BR.49
	Chlorine—neutralizing Ringer Solution to counteract the bactericidal effect of hypochlorites or other chlorine sources	Thiosulphate Ringer Tablets	BR.48

For details of Membrane Filtration Techniques, see the separate Oxoid Booklet

MEDIA FOR WATER AND SEWAGE MICROBIOLOGY

Groups of Micro-Organisms/Test	*Purpose*	*Media*	*Code*
Clostridium perfringens	Detection of *Clostridium perfringens* indicating remote or intermittent pollution in water	Iron Sulphite Agar	CM.79/80
		Reinforced Clostridial Medium	CM.149
	Confirmation of *Clostridium perfringens*		
	(a) by 'stormy-clot' reaction	Crossley Milk Medium	CM.213
	(b) by Nagler test	Blood Agar Base	CM.55
		Egg Yolk Emulsion	SR.47
		Fildes Extract	SR.46
Coliform Group (including *Esch. coli*)	Detection of coliforms or *Esch. coli* (at 44° C) as indicators of pollution		
	(a) Mean Probable Number Technique	Lactose Broth	CM.137
		Lauryl Tryptose Broth	CM.451
		MacConkey Broth (Purple)	CM.5a
		Minerals Modified Glutamate Medium	CM.289
	(b) Membrane Filtration Technique	MacConkey Membrane Broth	MM6a
		Membrane Enriched Teepol Broth	MM.369
		Resuscitation Membrane Broth	MM.20
	Confirmation of coliforms or *Esch. coli*	Brilliant Green Bile (2%) Broth	CM.31
		Endo Agar Base	CM.479
		Lactose Ricinoleate Broth	CM.371
		MacConkey Agar	CM.7
		MacConkey Agar No. 3	CM.115
		Violet Red Bile Agar	CM.107
	Differentiation of coliforms		
	(a) Methyl Red and Voges-Proskauer Tests	MRVP Medium	CM.43
	(b) Citrate Utilization	Simmons Citrate Agar	CM.155
	(c) Indole Production	Tryptone Water	CM.87
Faecal streptococci	Detection and isolation of faecal streptococci from sewage and water	Azide Blood Agar Base	CM.259
		MacConkey Agar No. 2	CM.109
		Slanetz and Bartley Medium	CM.377
Plate Count	Colony count for indicating the efficiency of water treatment processes, or suitability of water for food preparation. Nutrient gelatin is used for the plate count of psychrophilic organisms e.g. Pseudomonas species and for testing gelatinase activity	Nutrient Gelatin	CM.135a
		Standard Plate Count Agar (A.P.H.A.)	CM.463/464
		Tryptone Soya Agar	CM.131/132
		Yeast Extract Agar	CM.19
Salmonella and Shigella	Enrichment procedures prior to isolation	Mannitol Selenite Broth Base	CM.399
		Selenite Broth Base	CM.395
		Sodium biselenite	L.121
		Tetrathionate Broth Base	CM.29
	Isolation and identification of Salmonella and Shigella species	Bismuth Sulphite Agar	CM.201
		Brilliant Green Agar	CM.263
		Brilliant Green Agar (modified)	CM.329
		D.C.L.S. Agar	CM.393
		Desoxycholate Citrate Agar (Hynes)	CM.227
		Hextoen Enteric Agar	CM.419
		Kligler Iron Agar	CM.33/34
		Kohn Two-Tube Medium	CM.179/181
		Lysine Decarboxylase Broth	CM.308
		Lysine Iron Agar	CM.381
		Triple Sugar Iron Agar	CM.277/278
		XLD Medium	CM.469

MEDIA FOR WATER AND SEWAGE MICROBIOLOGY—*continued*

Groups of Micro-Organisms/Test	*Purpose*	*Media*	*Code*
Diluents used in water and sewage bacteriology	For preparation of ¼ strength Ringer solution, used as a diluent or rinse	Ringer Solution ¼ Strength Ringer Solution tablets	BR.52
	Preparation of a sodium hexametaphosphate Ringer solution, used as a solvent diluent solution in conjunction with calcium alginate swabs	'Calgon' Ringer Tablets	BR.49
	Preparation of chlorine-neutralizing Ringer Solution, used as a diluent or rinse after hypochlorites or other chlorine sources	Thiosulphate Ringer Tablets	BR.48

Membrane Filters, Incubating Tins and Pads are available from Oxoid. For details of Membrane Filtration Techniques for water and sewage, see the separate Oxoid Booklet

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OXOID PRODUCTS

Acid Egg Medium

Code PM1a

FORMULA

Potassium dihydrogen phosphate	*grams*	**12.3**
Magnesium sulphate		**0.3**
Glycerol		**12.0 ml**
Potato starch		**30.0**
Fresh egg mixture (yolks and whites)		**from 22 large eggs**
Malachite green		**0.4**
Sodium penicillin		**100,000 units**
Distilled water		**640 ml**

DESCRIPTION

A solid medium for use in a simplified method of *Mycobacterium tuberculosis* culture, described by Marks (1959). The method entails the use of an acid egg medium which may be inoculated with an alkaline sputum homogenate which has been neither neutralized nor centrifuged. This saves time, eliminates the need for an expensive centrifuge and reduces the risk of disseminating tubercle bacilli. The volume of medium (4.5 ml) was designed to neutralize the alkali in 0.2 ml of homogenate and this ratio should not be altered. Marks considered that his method was considerably more sensitive, and possessed a much lower contamination rate than those described by the International Union against Tuberculosis (1955) and by Nassau (1958), and summarizes 'With the conditions recommended, this method is considered to be 99% as effective as a full-scale method with film-positive specimens and 98% with film-negative specimens.'

TECHNIQUE (Marks, 1959)

1. Distribute 2 ml amounts of sterile 4% NaOH into sterile 1 oz. universal containers.

2. With a sterile plugged glass tube, pipette 2 ml amounts of sputum into the NaOH, avoiding contact with the top half of the bottles. With scanty specimens, first add sterile distilled water to the NaOH equivalent to the volume deficiency.

3. Shake the bottles briefly in a rack, avoiding contamination of the caps, and place in a 37° C dry incubator. Shake for 20 seconds after 7½, 15 and (if necessary) 22½ minutes. Inspect the mixtures after 15 and 22½ minutes, and each time immediately inoculate any which have become watery (ignore very small particles). Inoculate after 30 minutes' incubation, whatever the consistency. There is thus a minimum of 15 and a maximum of 30 minutes incubation during homogenisation.

4. On their withdrawal from the incubator, inoculate the alkaline homogenates directly into the Acid Egg Medium. For pipettes use sterile plugged glass tubes with an internal diameter of 4 mm and marked corresponding to 0.2 ml before sterilization. Pipettes may be re-used but because of the danger of false microscopical positives due to faulty cleaning, films of the sputum should be made before culturing.

Inoculate the Acid Egg Medium with 0.2 ml of homogenate and tilt so that the inoculum runs over the entire surface of the slope.

5. Incubate at 37° C and examine every week until the culture is discarded after a total of 8 weeks' incubation.

REFERENCES

Marks, J. (1959) 'A Simple Method for the Cultivation of Tubercle Bacilli' *Mon. Bull. Min. Hlth. Pub. Hlth. Lab. Srev.* **18**, 81–86.
Nassau, E. (1958) 'Sputum Swab Culture: Simple Method of Isolating Tubercle Bacili from Sputum' *Tubercle* **39**(1), 18–23.
Report (1955) *Bul. Int. Union Tuberc.* **25**, 89.

'Actidione' Agar

Code PM118

FORMULA

	grams per litre
Yeast Extract (Oxoid L20)	**4.0**
Tryptone (Oxoid L42)	**5.0**
Dextrose	**50.0**
Potassium dihydrogen phosphate	**0.55**
Potassium chloride	**0.425**
Calcium chloride	**0.125**
Magnesium sulphate	**0.125**
Ferric chloride	**0.0025**
Manganese sulphate	**0.0025**
Bromo-cresol green	**0.022**
'Actidione'	**0.01**
Agar No. 3 (Oxoid L13)	**15.0**

and distilled water to 1 litre
ph 5.5 (approx.)

DESCRIPTION

'Actidione' (cycloheximide, Upjohn Ltd.) at a concentration of 0.001% w/v permits the growth of bacteria but inhibits the growth of most yeasts and moulds except dermatophytes. Media containing this antibiotic are invaluable for the enumeration and detection of bacteria in specimens containing large numbers of yeasts and moulds. For example, the medium is used for the estimation of bacterial contamination of pitching yeast. 'Actidione' Agar with added penicillin and streptomycin is also valuable as a selective medium for the isolation of dermatophytes.

The Oxoid medium, based on that of Green and Gray (1950), may be used for microbiological investigations during brewing and baking. As an approximate guide, incubation may be at 25° C or 30° C for up to 14 days, according to the flora present. Green and Gray employed their medium at two different reactions, pH 5.5 and pH 6.5, the latter may be attained by adding approximately 16 ml of sterile 1.5% sodium carbonate to each litre of molten medium.

REFERENCE

Green, S. R. and Gray, P. P. (1950) *Wallerstein Lab. Communication* **13**, 357.

Oxoid Agars

Agar is the dried water-soluble extract from a group of red-purple seaweeds (rhodophyceae) which form the agarophyte group: Gelidium, Pterocladia, Gracilaria and Acanthopeltis species.

It is a sulphuric acid ester of a linear galactan molecule which is insoluble in cold water but soluble in hot water, a 1.5% w/v solution should set to a firm gel between 32° C—39° C and not melt below 85° C.
Although agar is a mixture of polysaccharides, Araki in 1937 divided them into two groups:

(i) low-sulphate, pyruvic acid-free *agarose*

(ii) high-sulphate, high ash *agaropectin*.

The proportion of agarose to agaropectin in agar varies according to the seaweed of origin but it can be as high as 75% agarose to 25% agaropectin. Approximately half of all the agar produced is used for microbiological work, here high gel strength and freedom from toxic metals are important. It should also be free from impurities commonly found in commercial agars: non-agar gums, nitrogenous compounds, insoluble salts, free sugars, dead micro-organisms and thermoduric bacteria.

The fastest growing agar-producing area in the world is around Spain, Portugal and Morocco. Oxoid played a major role in establishing standards of high quality agar for producers in this area. The high gel strength agars (1%—1.2% w/v) originated here and are now being adopted in the U.S. and elsewhere.

The technique of agar production has been fully described by Whistler (1973), Chapman (1970) and Bridson & Brecker (1970), but the products produced can be summarized as follows:

(a) commercial agar (sometimes called technical agar) produced primarily for the food industry, it is free from toxic substances but may contain high calcium and magnesium levels with relatively poor clarity.

(b) bacteriological agar which is specially clarified and free from pigments. The clarification process may not reduce the cation levels and phosphate 'floc' may appear in nutrient culture media after autoclaving.

(c) specially purified agars which have reduced ash and metal contents. These are compatible with all the normal ingredients of culture media and exhibit minimal inhibition of diffusion of antibiotics.

(d) separated agarose where a specific fraction of agar is collected and dried. Agarose preparations vary widely in chemical analysis but the specification should include electroendosmosis factors (m_r) below 0.15 for satisfactory gel electrophoresis of antibody/antigen complexes.

Oxoid agars cover all the requirements of microbiologists and are classified on the following page.

TECHNICAL INFORMATION

Average Batch Analysis	**L11**	**L13**	**L28**
Moisture	7.0%	14.6%	7.0%
Ash	2.0%	3.9%	1.2%
Acid Insoluble Ash	<0.1%	0.1%	<0.1%
Cl as NaCl	<0.01%	<0.01%	<0.01%
P as P_2O_5	<0.005%	<0.05%	<0.005%
SO_4	0.9%	1.9%	0.7%
Total Nitrogen	0.1%	0.1%	0.1%
Ca	150 ppm	6,000 ppm	200 ppm
Mg	100 ppm	2,000 ppm	100 ppm
Fe	80 ppm	200 ppm	60 ppm
Precipitate with Phosphates	None	Heavy	None
Working Strength	1.0%	1.2%	1.0%
M_r Index	0.32	0.4	0.23

- The Electroendosmosis value (m_r) of an agar gel is defined as the relative mobility of dextran to that of crystalline human albumin. The lower the figure the more suitable the agar for electrophoresis studies.

Agar Bacteriological (Agar No. 1)

Code L11

A specially purified bacteriological agar of very high gel strength (1% w/v) which has been processed to reduce its mineral content below that of any comparable agar on the market. Apart from its clarity and compatibility with all culture media, it is unique in that broth and agar formulations of the same medium can have very similar mineral contents. This is of extreme importance in antibiotic M.I.C. studies where minerals can play decisive roles. It is also the best agar for antibiotic diffusion studies as its low mineral content ensures that chemotherapeutic substances are not held back or complexed during their migration through the agar.

Agar Technical (Agar No. 3)

Code L13

A technical grade of high gel strength (1.2% w/v) which is suitable for those purposes where clarity and compatibility are not of prime importance.

Purified Agar

Code L28

An exceptionally bright and clear agar that has been specially processed to give a low m_r factor that is only exceeded by expensive agarose preparations. It is primarily recommended for electrophoresis studies but it is also suitable for normal bacteriological purposes.

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Agar Tablets

Code CM49 (Oxoid Agar No. 3)

DESCRIPTION

Compressed tablets of a pre-weighed amount of agar which are especially useful for solidifying liquid media or for the preparation of semi-solid media. Oxoid Agar Tablets are standardized to produce a satisfactory gel when dissolved in 10 ml of liquid. This eliminates batch to batch variation and the differences found between one type of agar and another. The use of these tablets obviates weighing and filtration—thus leading to considerable time saving.

TECHNIQUE

To produce a satisfactory gel for plate cultures and slopes, add 1 tablet to every 10 ml liquid medium and autoclave at 121° C for 15 minutes. With very acid media it is advisable to use 2 tablets to every 10–15 ml of medium. In order to prepare semi-solid agar use 1 tablet to every 100 ml of liquid medium.

Andrade Peptone Water

See page 217.

Antibiotic Assay Media

The methods and media required for the organisms used in the Bio-assay of Antibiotics are specified in the United States Pharmacopoeia, the British Pharmacopoeia and in other works of reference, e.g. Grove and Randall 'Assay Methods of Antibiotics'. To meet the requirements of workers in this field, 'Oxoid' produce the more widely used media in dehydrated form.

Antibiotic Medium No. 1

Seed Agar

Code CM327

FORMULA

	grams per litre
Peptone (Oxoid L37)	**6.0**
Tryptone (Oxoid L42)	**4.0**
Yeast Extract (Oxoid L20)	**3.0**
'Lab-Lemco' Powder (Oxoid L29)	**1.5**
Dextrose	**1.0**
Agar No. 1 (Oxoid L11)	**11.5**

pH 6.5 (approx.)

DIRECTIONS

Suspend 27 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This is perhaps the most important medium in antibiotic assay work and we have now introduced it in a specially modified form to take advantage of the properties of 'Oxoid' Agar No. 1.

In a paper published in 'Applied Microbiology' in 1967 (Vol. 15, No. 1, pp. 31–34) Hanus, Sands and Bennett drew attention to the inhibitory properties which certain agars have towards some antibiotics, particularly Streptomycin, Kanamycin, Polymyxin B and Neomycin. Because 'Oxoid' Agar No. 1 does not share these inhibitory properties it is especially suited to antibiotic assay work. In addition, this agar, with its superior diffusion properties, produces more clearly defined inhibition zones.

N.B.—IN THE 'OXOID' FORMULATION, ONLY 11.5 GRAMS OF AGAR NO. 1 ARE USED TO GIVE THE EQUIVALENT GEL STRENGTH OF 15 GRAMS ORDINARY AGAR.

This medium is used as a seed agar with Micrococcus flavus for the plate assay of Bacitracin; with Sarcina lutea for the plate assay of Chloramphenicol and with Staphylococcus aureus for the assay of Kanamycin sulphate, Penicillin G, Sodium Methicillin and Sodium Oxacillin.

It is also employed as a Base Agar in the assay of the following drugs: Chloramphenicol, Kanamycin Sulphate, Colistin Sulphate, Sodium Methicillin, Sodium Oxacillin and Vancomycin Hydrochloride.

REFERENCE

B.P. 1963, U.S.P. XVII.

Antibiotic Medium No. 2

Base Agar
Code CM335

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**6.0**
Yeast Extract Powder (Oxoid L21)		**3.0**
'Lab-Lemco' Powder (Oxoid L29)		**1.5**
Agar No. 1 (Oxoid L11)		**10.0**

pH 6.5 (approx.)

DIRECTIONS

Suspend 20.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This new formulation replaces our older product labelled 'Base Agar' which has been withdrawn.

Medium No. 2 is used as the Base Agar in the plate assay of Bacitracin and in the assay of Penicillin G.

REFERENCE

U.S.P. XVII.

Antibiotic Medium No. 3

Assay Broth
Code CM287

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**5.0**
Yeast Extract (Oxoid L20)		**1.5**
'Lab-Lemco' Powder (Oxoid L29)		**1.5**
Dextrose		**1.0**
Sodium chloride		**3.5**
Dipotassium hydrogen hhosphate		**3.68**
Potassium dihygrogen phosphate		**1.32**

pH 7.0 (approx.)

DIRECTIONS

Add 17.5 grams to 1 litre of warm (60° C) distilled water and mix well to dissolve. Distribute and sterilize by autoclaving for 15 minutes at 121° C.

DESCRIPTION

Medium No. 3 is used in the turbidimetric assay of Penicillin and Tetracycline with Staphylococcus aureus.

Taking advantage of modern technology, this medium is based on the original 'Penassay Broth' which has been withdrawn.

REFERENCE

U.S.P. XVII.

Antibiotic Medium No. 5

Streptomycin Assay Agar

This is very easily made by changing the pH of Antibiotic Medium No. 2. To one litre of Base Agar add 5 ml of normal caustic soda solution before autoclaving. This will bring the pH to 7.9, but no filtration will be necessary.

This medium is suitable for the streptomycin assay, using the cylinder plate technique and a test organism of Bacillus subtilis and gives clearly defined inhibition zones.

After this base layer has gelled, it is overlaid with the same medium already inoculated with spores of the test organism.

Antibiotic Medium No. 8

For the plate assay of Tetracycline and Vancomycin

This can readily be made by a small adjustment of the pH of Medium No. 2. To convert Medium No. 2 into Medium No. 8 aseptically add 1.25 ml of N/1 HCl, after sterilization, to the still molten Medium No. 2 at 50–55° C. This will bring the pH down to 5.6–5.7. No filtration will be necessary. Medium No. 8 is used for the base agar and seed agar for the plate assay of Tetracycline. It is also used as the seed agar for Vancomycin hydrochloride.

Antibiotic Medium No. 11

For the plate assay of Neomycin sulphate

This can readily be made by a small change of pH of Medium No. 1. To convert to Medium No. 11 aseptically add 5 ml of N/1 NaOH, after sterilization, to the still molten Medium No. 1 at 50–55° C. The pH will then be 7.9–8.0. No filtration will be necessary. Medium No. 11 is used as the base agar and seed agar for the plate assay of Neomycin sulphate.

Azide Blood Agar Base

Code CM259

FORMULA

	grams per litre
Tryptose (Oxoid L47)	**10.0**
'Lab-Lemco' Powder (Oxoid L29)	**3.0**
Sodium chloride	**5.0**
Sodium azide	**0.2**
Agar No. 3 (Oxoid L13)	**12.0**

pH 7.2 (approx.)

DIRECTIONS

Suspend 30 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. For azide blood agar, cool to 45–50° C and add 5% of sterile blood.

DESCRIPTION

A selective medium for the detection and isolation of streptococci from faeces, sewage, and other specimens containing a mixed flora. Azide Blood Agar Base is similar to the medium used by Edwards (1933) for the isolation of mastitis streptococci. The sodium azide has a bacteriostatic effect on most Gram-negative organisms but permits growth of Gram-positive organisms such as streptococci and some strains of staphylococci. *Proteus* species are slightly more resistant than other *Enterobacteriaceae* but swarming is prevented (Snyder and Lichstein, 1940; Lichstein and Snyder, 1941).

At the above concentration and pH, sodium azide exerts no appreciable effect on haemolysis—so that the medium, with added blood, may be used for the simultaneous determination of haemolytic reactions.

Azide blood agar is recommended by the American Public Health Association (1960) for the isolation of streptococci from cheese. The plates, inoculated with dilutions of emulsified cheese, are incubated at 35° C and representative colonies subcultured for subsequent identification.

A similar medium to Packer's (Packer, 1943) may be prepared by the aseptic addition of 4 ml of 0.05% aqueous crystal violet solution per litre of base after the addition of blood. Packer's crystal violet sodium azide blood agar is used for the enumeration of faecal streptococci (Mossel *et al.* 1957).

REFERENCES

American Public Health Association (1960) 'Standard Methods for the Examination of Dairy Products' 11th ed., APHA Inc., New York, p. 169 and 403.

Edwards, S. J. (1933) 'Studies on Bovine Mastitis. IX. A selective medium for the diagnosis of streptococcus mastitis' *J. Comp. Path. Therap.* **46**(4), 211–217.

Lichstein, H. C. and Snyder, M. L. (1941) 'The Inhibition of the Spreading Growth of *Proteus* and other Bacteria to Permit the Isolation of Associated Streptococci' *J. Bact.* **42**(5), 653–664.

Mossel, D. A. A. Diepen, H. M. J. van and De Bruin, A.S. (1957) 'The Enumeration of Faecal Streptococci in Foods using Packer's Crystal Violet Sodium Azide Blood Agar, *J. appl. Bact.* **20**(2), 265–272.

Packer, R. A. (1943) 'The Use of Sodium Azide (NaN_3) and Crystal Violet in a Selective Medium for Streptococci and *Erysipelothrix rhusiopathiae*' *J. Bact.* **46**, 343–349.

Snyder, M. L. and Lichstein, H. C. (1940) 'Sodium Azide as an Inhibiting Substance for Gram-Negative Bacteria' *J. infect. Dis.* **67**(2), 113–115.

Bacitracin Discs

Code DD2

DESCRIPTION

Sterile paper discs each containing 0.05 unit of bacitracin, for the differentiation of Lancefield Group A from other beta-haemolytic streptococci. When used as a screening test prior to serological grouping, Bacitracin Discs effect considerable economies in time, labour and materials.

Maxted (1953) showed that Group A streptococci were more sensitive to bacitracin than beta-haemolytic strains of other groups, and that bacitracin might therefore be used as a rapid diagnostic agent for Group A streptococci. He found that only 1.7% of 2,386 bacitracin sensitive strains proved not to be Group A, and only 2.5% of 851 resistant strains were Group A. Levinson and Frank (1955), who employed impregnated paper discs for the same purpose, observed that 93.2% of 866 sensitive beta-haemolytic streptococci belonged to Group A. Streamer *et al.* (1962) compared bacitracin disc, fluorescent antibody and Lancefield precipitin techniques for the identification of Group A streptococci. The bacitracin disc technique was considered to be the simplest and most practical for the routine clinical laboratory.

The use of bacitracin sensitivity testing is not restricted to the differentiation of beta-haemolytic streptococci. Guthof (1960) found sensitivity to optochin, bacitracin, and furacin to be a useful test for distinguishing *Aerococcus viridans* and *Streptococcus milleri* from enterococci and *mitis* streptococci.

TECHNIQUE

A. Pure Cultures

1. Evenly inoculate the surface of a Blood Agar Plate (Blood Agar Base No. 2 CM271 with 7% Sterile Oxalated Horse Blood SR49) with a pure culture of the beta-haemolytic streptococcus to be tested.

2. Aseptically place a Bacitracin Disc on the inoculated surface.

3. Incubate for 18 to 24 hours at 37° C.

4. Examine for the presence of a zone of inhibition around the Bacitracin Disc. A zone indicates that the streptococcus is presumptively Group A—if desired, further confirmation may be obtained by serological grouping.

B. Clinical Material

1. Inoculate a Blood Agar Plate with the throat swab or other material.

Spread the inoculum so as to ensure that discrete colonies occur on some portion of the plate—so that the species in a mixed growth may be determined.

2. Aseptically place a Bacitracin Disc on the secondary area of inoculation.

3. Incubate for 18 to 24 hours at 37° C.

4. Examine for zones of inhibition. Organisms other than beta-haemolytic streptococci are sensitive to bacitracin, so that the presence of a zone of inhibition does not necessarily indicate Lancefield Group A streptococci. It is possible to select presumptive Group A streptococci if attention is given to colonial morphology. Further confirmation may be obtained by serological grouping.

Bacitracin Discs DD2 are not recommended for routine testing of the sensitivity of organisms to Bacitracin. For this purpose it is advisable to employ Bacitracin Sensitivity Discs at a level of 10 units or Bacitracin (Code B) can be included in Special Multodisk combinations if required.

N.B.—BACITRACIN DISCS SHOULD BE STORED AT 4° C.

REFERENCES

Guthof, O. (1960) 'The Identity of Pathogenic Greening Streptococci with Normal Mouth Streptococci' *Ztschr. f. Hyg. u. Infektionskr.* **146**(5), 425–432.

Levinson, M. L. and Frank, P. F. (1955) 'Differentiation of Group A from other Beta Haemolytic Streptococci with Bacitracin' *J. Bact.* **69**(3), 284–287.

Maxted, W. R. (1953) 'The Use of Bacitracin for Identifying Group A Haemolytic Streptococci' *J. clin. Path.* **6**(3), 224–226

Streamer, C W. *et al.* (1962) 'Identification of Group A Streptococci, Bacitracin Disc and Fluorescent Antibody Techniques compared with the Lancefield Precipitin Method' *Amer. J. Dis. Children* **104**(2), 157–160.

Baird-Parker Medium

Code CM275

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**10**
'Lab-Lemco' Powder (Oxoid L29)		**5**
Yeast Extract (Oxoid L21)		**1**
Sodium pyruvate		**10**
Glycine		**12**
Lithium chloride		**5**
Agar No. 3 (Oxoid L13)		**20**

pH 6.8 (approx.)

DIRECTIONS

Suspend 63 g in one litre of distilled water and boil to dissolve the medium completely. Dispense into tubes or flasks and sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C and aseptically add 50 ml of Egg Yolk-Tellurite Emulsion (SR54). Mix well before pouring.

PREPARED PLATES MAY BE STORED AT 4° C.

DESCRIPTION

This highly specific diagnostic and selective medium was developed by Baird-Parker (1962) for the isolation and enumeration of coagulase-positive staphylococci from food. The medium was developed because of the unreliability of published methods for the isolation of *Staphylococcus aureus* and because a more specific medium was needed on which this organism could be isolated within 24 hours. The tellurite glycine medium of Zebovitz *et al.* (1955) was modified by Baird-Parker who then added sodium pyruvate as a selective growth stimulant and Oxoid Concentrated Egg Yolk Emulsion as a diagnostic agent. Baird-Parker Medium is not inhibitory to *Staphylococcus aureus* and yet is sufficiently selective to suppress the growth of most other bacteria occurring in food. The few organisms which do occasionally grow (e.g. *Staphylococcus saprophyticus*) produce distinctive colonies which do not give rise to confusion. Staphylococci show two diagnostic characteristics on the opaque medium: Firstly they may produce clear zones by proteolysis. Secondly opaque zones may appear within the clear zones (probably caused by a lipase). *Staphylococcus aureus* and some strains of *Staphylococcus saprophyticus* (Shaw *et al.*, 1951) may produce both of these characteristics but they are easily distinguished from each other by the different times at which the opacity develops. The medium permits the detection of very small numbers of *Staphylococcus aureus*, and has shown a much higher correlation with the tube test for coagulase than four other published media (Baird-Parker, 1962). Holbrook *et al.* (1969) found that Baird-Parker Medium plates could withstand prolonged storage if the pyruvate was added just before use. The Oxoid stabilised Egg Yolk-tellurite emulsion overcomes this problem and prepared plates may be stored at 4° C for up to 4 weeks before use.

Sharpe *et al.* (1962) compared Baird-Parker medium with other media, for the isolation of staphylococci from raw milk and cheese. On mannitol salt agar only 49.9% of the total number of colonies from all samples were coagulase positive. On phenolphthalein phosphate agar 54.9% of phosphatase positive colonies were coagulase positive. With Baird-Parker medium 85.2% of presumptive coagulase positive staphylococci were proved to be so on testing. When an experienced worker counted colonies showing a typical appearance on Baird-Parker medium, it was seldom necessary to confirm the results by an actual coagulase test.

Smith and Baird-Parker (1964) found that the addition of 50 μg of sulphamethazine per ml of medium suppressed the growth and swarming of *Proteus* species. Small numbers of *Staphylococcus aureus* could then be recovered from specimens containing mixed *Proteus* strains.

Baird-Parker and Davenport (1965) showed that the recovery of damaged staphylococci was greater on Baird-Parker medium than on other recovery media tested.

Broeke (1967) and de Waart *et al.* (1968) found Baird-Parker medium valuable in ecological studies on foods incriminated in staphyloenterotoxicosis. 97.5% of the 522 strains of Staph. aureus tested, isolated from human and food origins, developed characteristically and quantitatively on Baird-Parker medium.

TECHNIQUE

1. Dry the surface of prepared plates.

2. With a glass spatula, spread from 0.1–1.0 ml of dilutions of food (macerated in 0.1% peptone water) over the surface of each well dried plate.

3. Incubate for 24–26 hours at 37° C and examine. Incubate for a further 24 hours if no *Staphylococcus aureus* colonies are seen.

COLONIAL APPEARANCES

Staphylococcus aureus—after 24 hours, black, shiny, convex, 1.0–1.5 mm diameter, narrow white entire margin and surrounded by a zone of clearing 2–5 mm in width. Some strains show well defined clearing only after 36 hours. May produce wide opaque zones extending into the cleared medium but only after 48 hours or longer. Coagulase-negative staphylococci—occasionally grow, but seldom produce clearing. *Staphylococcus saprophyticus* may produce clearing but colonies are more irregular than *S. aureus*. Wide opaque zones extend into the cleared medium after 24 hours. Micrococci—occasionally grow, minute, various shades of brown or black, no clearing.

Yeasts—occasionally grow, white, no clearing.

Bacillus species—occasionally grow, sometimes clear medium after 48 hours but have dark brown matt colonies.

REFERENCES

Baird-Parker, A. C. (1962) 'An Improved Diagnostic and Selective Medium for Isolating Coagulase Positive Staphylococci' *J. appl. Bact.* **25**(1), 12–19.

Baird-Parker, A. C. and Davenport, E. (1965) 'The Effect of Recovery Medium on the Isolation of *Staphylococcus aureus* after Heat Treatment and after the storage of Frozen or Dried Cells' *J. Appl. Bact.* **28**, 390–402.

Broeke, R. Ten (1967) 'The Staphylococcus Medium of Baird-Parker in Practical Use. The Occurrence of Coagulase Positive, Egg Yolk-Non Clearing Staphylococci' *Antonie van Leeuwenhoek* **33**, 220.

Holbrook, R., Anderson, Judy M. and Baird-Parker, A. C. (1969) 'The Performance of a Stable Version of Baird-Parker's Medium for Isolating *S. aureus*' *J. Appl. Bact.* **32**, 187–192.

Sharpe, M. Elisabeth, Neave, F. K. and Reiter, B. (1962) 'Staphylococci and Micrococci Associated with Dairying' *J. appl. Bact.* **25**(3), 403–415.

Shaw, Constance, Stitt, Jennifer M. and Cowan, S. T. (1951) 'Staphylococci and their Classification' *J. gen. Microbiol.* **5**(5), 1010–1023.

Smith, B. A. and Baird-Parker, A. C. (1964) 'The Use of Sulphamethazine for Inhibiting *Proteus* Spp. on Baird-Parker's Isolation Medium for *Staphylococcus aureus*' *J. appl. Bact.* **27**(1), 78–82.

Waart, J. de, Mossel, D. A. A., Broeke, R. Ten and Moosdijk, A. van de (1968) 'Enumeration of *Staphylococcus aureus* in Foods with Special Reference to Egg Yolk Reaction and Mannitol Negative Mutants' *J. Appl. Bact.* **31**, 276–285.

Zebovitz, E., Evans, J. B. and Niven, C. F. (Jr.) (1955) 'Tellurite-Glycine Agar: A Selective Plating Medium for the Quantitative Detection of Coagulase-Positive Staphylococci' *J. Bact.* **70**, 686.

Barbitone Acetate Buffer for Electrophoresis

Code BR11g—

FORMULA

Sodium acetate	*grams per litre*	**6.5**
Sodium barbitone (barbitone soluble)		**8.87**
Barbitone		**1.13**

pH 8.6

DIRECTIONS

To prepare a buffer solution with ionic strength 0.1 and pH 8.6, dissolve 16.5 grams in 500 ml of warm distilled water, cool, make up to 1 litre with distilled water.

DESCRIPTION

This product is supplied in powder form for the rapid preparation of a buffer solution for use in cellulose acetate and filter paper strip electrophoresis.

In his paper on electrophoresis Owen (1956) give the formula of a buffer similar to that described by Michaelis (1931). A modification has been developed in the Oxoid Laboratories whereby fresh buffers may be prepared by dissolving a weighed amount of granules in water. Thus the need for titration with hydrochloric acid has been eliminated.

REFERENCES

Michaelis, L. (1931) 'The Acetate-Barbital Buffer' *Biochem Z.* **234**, 139–141.

Owen, J. A. (1956) 'Determination of Serum-Protein Fractions by Zone Electrophoresis on Paper and Direct Reflection Photometry' *Analyst* **81**, 26–37.

Barbitone CFT Diluent Tablets

See page 95

Bile Salts

Code L55

A standardized bile extract, consisting mainly of sodium glycocholate and sodium taurocholate, for use as a selective inhibitory agent in bacteriological culture media such as MacConkey Agar CM7 and MacConkey Broth CM5. Bile Salts L55 conforms to bacteriological requirements and batches are standardized, with respect to inhibitory properties, by the method of Burman (1955). It is generally employed in culture media at a concentration of 5 grams per litre. Bile Salts L55 meets the following specification:

Appearance—A 2% aqueous solution is light straw coloured, clear and free from extraneous matter.

Reaction (2% aqueous solution)—pH 6.0 ± 0.2.

Minimal Effective Concentration (Burman, 1955)—0.25%–0.5%.

Bacteriological Performance—MacConkey broth and agar made up with L55 at the minimal effective concentration (MEC) support satisfactory growth of bile-tolerant organisms including pathogenic staphylococci. Gas production by clostridia in broths containing 0.5% of L55 is inhibited when the concentration is raised to 2%, as in Brilliant Green Bile (2%) Broth CM31 thus eliminating false positives in the 44° C test for Escherichia coli I. During the testing of milk for *Escherichia coli* I with Brilliant Green Bile (2%) Broth, false positives due to milk lactobacilli do not occur if the medium contains L55. An average batch analysis is:

Water	3.2%
Ash	19.3%
Chlorine (as NaCl)	3.6%
Copper	6 ppm
Iron	15 ppm

REFERENCE

Burman, N. P. (1955) 'The Standardization and Selection of Bile Salts and Peptone for Culture Media used in the Bacteriological Examination of Water' *Proc. Soc. Water Treat. Exam.* **4**, 10.

Bile Salts No. 3

Code L56

Oxoid Bile Salts No. 3 was developed to meet the demand for a refined bile salt for use as a selective inhibitory agent in bacteriological culture media. It consists of a specially modified fraction of bile acid salts which is effective at less than one-third of the concentration of bile salts normally quoted. In selective media such as MacConkey Agar No. 3, Salmonella Shigella Agar and Violet Red Bile Agar the optimum concentration of Bile Salts No. 3 is 0.15% w/v. In such media there is a very sharp differentiation between lactose fermenters and non-lactose fermenters of enteric origin-permitting the detection of scanty non-lactose fermenters in the presence of numerous coliforms.

Bile Salts No. 3 L56 meets the following specification:

Appearance—A 0.15% aqueous solution is colourless, clear and free from extraneous matter.

Reaction (0.15% aqueous solution)—pH 7.6 ± 0.2.

Minimal Effective Concentration (Burman, 1955)—0.1–0.2%.

Bacteriological Performance—MacConkey Agar No. 3 made up with L56 supports satisfactory growth of test organisms, whilst Gram-positive cocci are suppressed.

An average batch analysis is:

Water	1.8%
Ash	17.6%
Chlorine (as NaCl)	1.5%
Copper	1–2 ppm
Iron	10 ppm

REFERENCES

Burman, N. P. (1955) 'The Standardization and Selection of Bile Salts and Peptone for Culture Media used in the Bacteriological Examination of Water' *Proc. Soc. Water Treat. Exam.* **4**, 10.

Bismuth Sulphite Agar

Wilson and Blair—modified
Code CM201

FORMULA

	grams per litre
'Lab-Lemco, Powder (Oxoid L29)	**5.0**
Peptone (Oxoid L37)	**10.0**
Dextrose	**5.0**
Disodium phosphate	**4.0**
Ferrous sulphate	**0.3**
Bismuth sulphite indicator	**8.0**
Brilliant Green	**0.025**
Agar No. 3 (Oxoid L13)	**20.0**

pH 7.7 (approx.)

DIRECTIONS

Suspend 5.2 g in 100 ml of cold distilled water in a 250 ml flask. For larger volumes of medium use a proportionately larger flask to ensure adequate space for mixing during heating. Any restriction of mixing during heating leads to superheating with reduction of the bismuth indicator to sulphide, and to caramelization of the dextrose. DO NOT RECONSTITUTE MORE THAN 400 ml. OF MEDIUM IN ONE FLASK.

Then place the flask on a tripod and gauze over a Bunsen flame and heat, with frequent agitation, until the medium froths up towards the neck of the flask. Remove the flask from the gauze to allow the froth to subside, withdraw the flame from under the gauze, replace the flask on the hot gauze and allow the contents to simmer for a half to one minute to dissolve the agar. Complete solution is indicated by the absence of flecks or 'tears' of molten agar from the sides of the flask.

The selectivity of the medium depends largely upon the uniform dispersion of precipitated bismuth sulphite in the final gel—for this reason the medium must be kept well mixed and should not be poured whilst too hot: hot plates tend to allow the precipitate to settle during gelling of the agar. Cool to 50–55° C, agitate to disperse the precipitate evenly, then immediately pour approximately 20 ml into each 10 cm (4 in.) Petri dish. Thin plates (8–10 ml per dish) are prone to desiccation and give retarded growth with inhibited staining by sulphide producing colonies due to concentration of ingredients.

Reconstitution not only dissolves the ingredients but causes precipitation of the bismuth sulphite which is an integral part of the medium. Poured and gelled plates should present a smooth cream-like opacity, and should be of a pale green colour free from blackening, with no sedimentation of the indicator. The freshly prepared medium is in a reduced state but gradually oxidises on cooling and storage: this is shown by the cream brown colour of the hot molten agar, which slowly turns pale green on gelling and finally becomes deeper green after storage.

Due to its content of reactive and hygroscopic substances, dehydrated Bismuth Sulphite Agar quickly deteriorates when exposed to the atmosphere. This is usually indicated by aggregation into a solid non-friable mass, and by the development of a brown coloration. Medium reconstituted from such material is brown, does not become green on storage, and is characterized by loss of differential and selective properties. For this reason the powder should be stored in a cool dry place and, after use, the container should be tightly closed.

DESCRIPTION

Bismuth Sulphite Agar is a modification of the original Wilson and Blair selective medium* for the isolation and preliminary identification of *Salmonella typhosa* (*Salmonella typhi*) and other salmonellae from pathological material, sewage, water supplies, food, and other products suspected of containing these pathogens.

In this medium freshly precipitated bismuth sulphite acts together with brilliant green as a selective agent, by suppressing the growth of coliforms whilst permitting the growth of salmonellae. Sulphur compounds provide a substrate for hydrogen sulphide production, whilst the metallic salts in the medium stain the colony and surrounding medium black or brown in the presence of hydrogen sulphide.

For some salmonellae, e.g. *Salmonella typhimurium*, the freshly poured medium is considered to be too inhibitory and Cook (1952) recommended storage of Bismuth Sulphite Agar Plates at 4° C for 4 days prior to use.

Atypical colonies may appear if the medium is too heavily inoculated with a specimen containing much organic matter. This may be prevented if the inoculum is prepared by mixing the specimen with sterile water or saline in a test tube. A plug of cotton-wool is then forced through the mixture with a glass rod. The fluid which exudes through the plug, free from gross particles, is used to inoculate plates of the medium.

Where the number of salmonellae is scanty, enrichment methods may be employed. Membrane filtration techniques are described in a separate booklet—see page 184.

COLONIAL APPEARANCES

Storage of the medium for 4 days at 4° C will often decrease the necessary incubation time and is considered by some to be essential for the production of characteristic *Salmonella* colonies (Cook, 1952). The medium may be read after 18 hours of incubation at 37° C but colonies showing a black zone of sulphide staining do not always show a metallic sheen until a few hours later. Both sulphide staining and metallic sheen may become more obvious if the incubated plates are left on the bench, at room temperature and in the light, for 2–3 hours.

All 'negative' plates should be incubated for 48 hours.

It is particularly important with Bismuth Sulphite Agar to spread the inoculum so that well isolated colonies are obtained on some portion of the plate.

REFERENCES

Cook, G. T. (1952) 'Comparison of Two Modifications of Bismuth-Sulphite Agar for the Isolation and Growth of *Salmonella typhi* and *Salm. typhimurium*' *J. Path. Bact.* **64**(3), 559–566.

Ministry of Health: Public Health Laboratory Service Water Committee (1969) Report No. 71: 'The Bacteriological Examination of Water Supplies' 4th ed., HMSO, London.

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* Do not confuse with the Wilson and Blair medium for the isolation of Clostridium perfringens (Ministry of Health, 1956).

The following are typical colonial appearances:

	Remarks	*Colour*	*Size*	*Black Zone Sulphide Stain Round Colony*	*Metallic Sheen**
Salmonella typhosa (*Salmonella typhi*)	'Rabbit-eye' colony	Black centre with clear translucent edge—uniform black after 48 hours		+ (18–24 hours)	+ (18–24 hours)
Other salmonellae		Black, green or clear	Often smaller and slower to develop than *S. typhosa*	± (36–48 hours)	± (36–48 hours)
Other organisms	Occasional strains of Coliforms, shigellae and Proteus	Green, brown, black	Usually smaller than *S. typhosa*	Usually –	Usually –

+ Positive — Negative ± Variable

* Should always be present when there is a zone of sulphide staining but may not be apparent till later. Massed colonies invariably do not develop these characteristics but appear geeen.

Blood Agar Media

Oxoid manufactures a wide range of products for use in the preparation of Blood Agar Media. These products are listed below and full details of each can be found in this manual alphabetically:

- Blood Agar Base-CM 55
- Blood Agar Base No. 2-CM 271
- Columbia Blood Agar Base-CM 331
- Diagnostic Sensitivity Test Agar-CM 261
- Tryptone Soya Agar-CM 131
- Tryptose Blood Agar Base-CM 233
- Blood Products (Horse Blood—Oxalated or Defibrinated-SR49/50)

Blood Products

Horse Blood, Defibrinated (No Preservative) *Code SR50*

Horse Blood, Oxalated (No Preservative) *Code SR49*

Sheep Blood, Defibrinated (No Preservative) *Code SR51*

Defibrinated and oxalated horse blood, and defibrinated sheep blood are employed for incorporation in bacteriological culture media, as sources of nutrients and indicators of haemolysis. Due to variation in the natural product, all are routinely tested for sterility, haemoglobin concentration, packed cell volume, and for compatibility and stability with culture media.

All operations are carried out under the strictest aseptic conditions, but in view of the very short time between bleeding the animals and delivery to the customer it is not possible to guarantee sterility since results of sterility tests are not immediately available.

Choice of blood is governed by the exact purpose of the investigation for it is well known that one particular species of micro-organism may exhibit dissimilar haemolytic reactions with different types of blood.

Defibrinated blood lyses more easily than oxalated blood (Stokes, 1955) and so is more sensitive for the investigation of haemolytic properties. However, defibrinated horse blood is so nutritive for *Haemophilus* that satellitism may not be seen and oxalated horse blood is preferred (Stokes, 1955).

Horse blood is most commonly employed in blood media, especially blood agar (e.g. Cruickshank, 1960; Wilson and Miles, 1957) but for certain purposes it may be preferable to employ sheep blood. For instance, sheep cells are often strongly haemolysed by pseudoanthrax bacilli, but only weakly haemolysed by *Bacillus anthracis*. Conversely, *Clostridium botulinum* which also does not haemolyse sheep blood, produces alpha-prime haemolysis on horse blood agar (Wilson and Miles, 1957). Sheep blood is employed in Edwards Medium—see page 118.

REFERENCES

Cruickshank, R. (1960) 'Mackie and McCartney's Handbook of Bacteriology' 10th ed., Livingstone Ltd., London.

Stokes, E. Joan (1955) 'Clinical Bacteriology' Edward Arnold Ltd., London.

Wilson, G. S. and Miles, A. A. (1957) 'Topley and Wilson's Principles of Bacteriology and Immunity' 4th ed., Edward Arnold Ltd., London, vol. 1.

Horse Blood, Laked

Code SR48

Horse blood lysed with saponin, for addition to Hoyle Medium Base (see page 112) and other laked blood media such as Monckton enrichment medium. Young (1942) investigated the use of saponin as a haemolysing agent in Hoyle medium. He found that it could be used in place of freezing and thawing, and was not inhibitory to *Corynebacterium diphtheriae*.

REFERENCE

Young, M. Y. (1942) 'Diphtheria Diagnosis with Hoyle's Medium. Saponin and Sodium-Dioctyl-Sulpho-Succinate as haemolysing agents in the preparation of the medium' *J. Path. Bact.* **54**, 253–256.

Sheep Blood Defibrinated

(FORMOLIZED)

Code SR52

A standardized product for the preparation of the sheep cell suspensions employed in immunological and serological techniques such as the Kolmer (Levinson and MacFate, 1956) and Wassermann (Cruickshank, 1960) complement-fixation tests for syphilis, the Rose-Waaler test for rheumatoid arthritis (Cruickshank, 1960), and the Paul-Bunnell reaction for infectious mononucleosis (Cruickshank, 1960).

REFERENCES

Cruickshank, R. (1960) Mackie and McCartney's Handbook of Bacteriology 10th ed., Livingstone Ltd., London.

Levinson, S. A. and MacFate, R. P. (1956) 'Clinical Laboratory Diagnosis 6th ed., Henry Kimpton, London.

Sheep Blood In Alsever Solution

Code SR53

A 50% concentration of sheep blood preserved in Alsever solution, for use in complement fixation tests such as the Wasserman reaction and virological complement fixation techniques. The preparation remains suitable for use up to 4 weeks if stored at 4° C.

Blood Agar Base

Code—Powder CM55
Tablets CM56

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**10**
Peptone (Oxoid L37)		**10**
Sodium chloride		**5**
Agar No. 3 (Oxoid L13)		**15**

pH 7.3 (approx.)

DIRECTIONS

Powder: Suspend 40 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving for 15 minutes at 121° C.

For blood agar, cool the Base to 50° C and add 7% of Defibrinated Horse Blood SR50. Mix with gentle rotation and pour into Petri dishes (12 ml for a 9 cm dish) or other containers.

DESCRIPTION

Oxoid Blood Agar Base is a non-selective general purpose medium widely employed for the growth of pathogenic and non-pathogenic bacteria:

(i) *Without additions,* the medium may be employed as a nutrient agar (a richer medium than Nutrient Agar CM3), or as a medium for the short-term maintenance of stock cultures.

(ii) *With added serum or other enrichments,* the medium becomes suitable for the cultivation of many fastidious organisms. Serum and other thermolabile enrichments should be added to the sterilized medium cooled to 45–50° C.

(iii) *With added blood,* the medium is not only enriched, but becomes suitable for the determination of the typical haemolytic reactions which are important diagnostic criteria for streptococci, staphylococci, and other organisms. For blood agar, 7% of sterile blood should be added to the sterilized medium cooled to 45–50° C.

Alper and Gillies (1960) and Hodgkins and Alper (1963) employed Oxoid blood agar base during investigations on irradiated *Escherichia coli* and other bacteria. Williams Smith (1959) found a reinforced Oxoid blood agar base was the most suitable medium for investigating the phages of *Clostridium perfringens* whilst Noble (1961) used Oxoid blood agar base as the basis of a selective medium for *Clostridium perfringens.* Noble (1962) used the medium, with added phenolphthalein phosphate, for the detection of phosphatase producing staphylococci. Hansen (1962) employed Oxoid blood agar base, with added salt and agar, for the assessment of surface contamination on equipment and pig carcases. Hayes (1963) determined the salinity range of growth of marine flavobacteria, using Oxoid blood agar base.

REFERENCES

Alper, T. and Gillies, N. E. (1960) 'The Relationship between Growth and Survival after Irradiation of *Escherichia coli* Strain B and two Resistant Mutants' *J. gen. Microbiol.* **22**(1), 113–128.
Hansen, N.-H. (1962) 'A Simplified Method for the Measurement of Bacterial Surface Contamination in Food Plants and its use in the Evaluation of Pressure Cleaners' *J. appl. Bact.* **25**(1), 46–53.
Hayes, P. R. (1963) 'Studies on Marine Flavobacteria' *J. gen. Microbiol.* **30** (1), 1–19.
Hodgkins, Brenda and Alper, T. (1963) 'Anaerobic Growth as a Factor Influencing Radio-sensitivity' *J. gen. Microbiol.* **30**(2), 307–315.
Noble, W. C. (1961) 'The Size Distribution of Airborne Particles carrying *Clostridium welchii* *J. Path. Bact.* **81**(2), 523–526.
Noble, W. C. (1962) 'The Dispersal of Staphylococci in Hospital Wards' *J. clin. Path.* **15**(6), 552–558.
Williams Smith, H. (1959) 'The Bacteriophages of *Clostridium perfringens*' *J. gen. Microbiol.* **21**(3), 622–630.

Blood Agar Base No. 2

Code CM271

FORMULA

Proteose Peptone (Oxoid L46)	*grams per litre*	**15.0**
Liver Digest (Oxoid L27)		**2.5**
Yeast Extract (Oxoid L20)		**5.0**
Sodium chloride		**5.0**
Agar No. 3 (Oxoid L13)		**12.0**

pH 7.4 (approx.)

DIRECTIONS

Suspend 40 g in 1 litre of distilled water, bring to the boil to dissolve completely. Mix and sterilize by autoclaving at 121° C for 15 minutes. Cool to 45–50° C and add 7% sterile blood.

Mix with gentle rotation and pour into Petri dishes (12 ml for a 9 cm dish) or other containers. RECONSTITUTION AND MIXING SHOULD BE PERFORMED IN A FLASK AT LEAST 2½ TIMES THE VOLUME OF MEDIUM TO ENSURE ADEQUATE AERATION OF THE BLOOD.

DESCRIPTION

Oxoid Blood Agar Base No. 2 was developed to meet the demand for an especially nutritious blood agar base which would permit the maximum recovery of delicate organisms without interfering with their haemolytic reactions. In comparison with fresh digest agar, Blood Agar Base No. 2 may be shown to have equal or superior growth promoting properties and chromogenic bacteria grown on the Oxoid medium show enhanced pigment formation. Comparison with many other blood agars has shown that with Oxoid Blood Agar No. 2 growth of many bacteria—especially the fastidious streptococci and pneumococci—is considerably improved, as shown by luxuriant and early colonial development.

The medium with added nutrients and selective agents is especially suitable for the isolation of *Mycoplasma* (PPLO) species: add 40 grams of Blood Agar Base No. 2 powder, 5 g of dextrose and 0.125 g of thallous acetate or thallous sulphate to 1 litre of distilled water and boil to dissolve. Mix, and sterilize by autoclaving at 121° for 15 minutes. Cool to

50° C, add 100,000 units penicillin and 200 ml of Horse Serum SR35, mix and pour thick plates. Inoculate and incubate separate plates for up to 5 or 6 days at 37° C under aerobic and anaerobic conditions respectively.

For the primary isolation of *Haemophilus* species from specimens containing a mixed flora, use Blood Agar Base No. 2 with added Defibrinated Horse Blood SR50. Even better results may be obtained using the horse blood agar plates with half of each spread with 2 drops of 10% saponin (Waterworth, 1955). Where haemolytic reactions are not important, for example when dealing with pure cultures, the Base may be used to prepare 'chocolate' agar: Add 10% of Defibrinated Horse Blood SR50 to the Base at 80° C and maintain at this temperature for 5 to 10 minutes, agitating frequently. Cool to 50° C, mix well and pour plates.

REFERENCE

Waterworth, Pamela M. (1955) 'The Stimulation and Inhibition of the Growth of *Haemophilus Influenzae* on Media containing Blood' *Brit. J. Exp. Path.* **36**(2), 186– 194.

Bordet-Gengou Agar Base

Code CM267

FORMULA

Potato Infusion . . . . . . *grams per litre*	**4.5**
Proteose Peptone (Oxoid L46)	**10.0**
Sodium chloride	**5.5**
Agar No. 3 (Oxoid L13)	**16.0**

pH 6.7 (approx.)

DIRECTIONS

Suspend 36 g in 1 litre of 1% solution of glycerol in distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 45–50° C and aseptically add 150–200 ml of sterile fresh defibrinated horse blood (warmed to 37° C). Mix thoroughly, taking care to avoid incorporation of air bubbles, and distribute.

The medium may be rendered selective by the aseptic addition of 0.25 units of penicillin per millilitre.

DESCRIPTION

Bordet-Gengou Agar Base with added glycerol and blood is used for the detection and isolation of *Bordetella pertussis* and *Bordetella parapertussis*. These organisms grow in the smooth phase on the Oxoid medium. The medium is similar to that used by Bordet and Gengou (1906) for the original isolation of *B. pertussis*, but the Oxoid medium is enriched with peptone and so is also suitable for the mass culture of *B. pertussis* as in vaccine production. Tarshis and Frisch (1951) cultivated *Mycobacterium tuberculosis* on Bordet-Gengou medium containing 25% of blood and 1% of glycerol.

TECHNIQUE

1. Distribute 30–40 ml of complete medium per 9–10 cm Petri dish. The poured plates should be bright cherry red and should not be dried in an incubator. Plates not used immediately may still be used after storage for several days in a refrigerator—provided they maintain a bright cherry red colour and remain moist.

2. Use two Bordet-Gengou plates, one containing penicillin, for each patient. Inoculate the plates by holding them approximately 10 cm in front of the patient's mouth whilst coughing. Alternatively, inoculate a small portion of the penicillin-free plate with a nasopharyngeal or preferably a pernasal swab (Wilson and Miles, 1957) and use a platinum loop to spread the inoculum over the remainder of the plate. Smear the swab over the whole of the penicillin-containing plate.

3. Incubate the plates at 37° C in a moist chamber to prevent desiccation of the medium.

4. Examine the plates for *B. pertussis* colonies after 40 hours and twice daily thereafter, and discard negative plates after 6 days' incubation. *B. pertussis* colonies at 48–72 hours are less than 1 mm in diameter, smooth, raised, glistening. They are almost transparent with a 'bisected pearl' appearance and surrounded by a zone of haemolysis with an indefinite periphery. *B. parapertussis* colonies are similar but grow more rapidly (i.e. are larger and well developed at 48 hours) and the underlying medium is green-black. Colonies of Gram-positive cocci are generally duller, darker, and more opaque.

5. Identify suspect colonies by serological and other methods (e.g. American Public Health Association, 1963).

REFERENCES

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Tarshis, M. S. and Frisch, A. W. (1951) *Am. J. clin. Path.* **21**, 101.113.
Wilson, G. S. and Miles, A. A. (1964) 'Topley and Wilson's Principles of Bacteriology and Immunity' 5th ed., Edward Arnold Ltd., London, p. 2011.

Bovine Serum

Code SR33

Sterile unheated bovine serum for use in bacteriological culture media. Bovine Serum is obtained from blood which has been allowed to clot naturally. Oxoid Bovine Serum does not contain additives.

Brain Heart Infusion

Code—Powder CM225
Tablets CM226

FORMULA

Calf Brain infusion solids . . . *grams per litre*	**12.5**
Beef Heart infusion solids	**5.0**
Proteose Peptone (Oxoid L46)	**10.0**
Dextrose	**2.0**
Sodium chloride	**5.0**
Disodium phosphate	**2.5**

pH 7.4 (approx.)

DIRECTIONS

Powder: Add 37 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 121°C for 15 minutes.

DESCRIPTION

A versatile liquid infusion medium which is suitable for the cultivation of streptococci, pneumococci, meningococci, and other fastidious organisms. This medium is recommended for blood culture work and, with the additions described below, for the isolation and cultivation of pathogenic fungi.

Oxoid Brain Heart Infusion is essentially a buffered infusion broth giving similar results to the brain dextrose broths originally employed by Rosenow (1919) for the cultivation of streptococci, and by Hayden (1923) for the cultivation of dental pathogens.

The addition of 0.1% of agar will serve to reduce convection currents and so create conditions of varying oxygen tension which favour the growth and primary isolation of aerobes and anaerobes (Hitchens, 1921), while even easily cultivated organisms show improved growth (Falk *et al.*, 1939).

Chapman *et al.* (1944) and Chapman (1946) used brain heart infusion in their test for the pathogenicity of streptococci, while Reitzel and Kohl (1938) enriched the same medium with ascitic fluid for the cultivation of gonococci.

Oxoid Brain Heart Infusion is especially useful as a growth and suspension medium for staphylococci which are to be tested for coagulase production; Newman (1950) employed a similar medium for this purpose in an investigation of food poisoning caused by dairy products.

Roseburg *et al.* (1944) added 2% of agar to brain heart infusion for the isolation and propagation of *Actinomyces israeli*, whilst Conant (1950) employed a similar medium for the cultivation of *Actinomyces bovis.*

Brain Heart Infusion with the addition of agar and 10% of Defibrinated Horse Blood SR50 supports the growth of *Histoplasma capsulatum.* With the further addition of 20 units of penicillin and 40 units of streptomycin per ml, the medium is known as Thompson agar and is suitable for the isolation of *Histoplasma capsulatum* (Howell, 1948) and other fungi, including *Coccidioides immitis* (Creitz and Puckett, 1954).

In order to prepare a satisfactory medium for blood culture, add 1 Agar Tablet CM49 to each 100 ml of Brain Heart Infusion and sterilize by autoclaving for 15 minutes at 121° C. Cool to 60–70° C and mix gently to ensure uniform distribution of the agar.

Tubes of Brain Heart Infusion which are not used the same day as sterilized should be placed in a boiling water bath for several minutes to remove absorbed oxygen, and cooled rapidly without shaking, just before use.

REFERENCES

Chapman (1946) *Am. J. Digestive Diseases* **13,** 105-107.
Chapman *et al.* (1944) *Am. J. clin. Path.* **9:** Tech. Suppl. 3, 20-26.
Conant, N. F. (1950) 'Diagnostic Procedures and Reagents' 3rd ed., American Pub. Hlth Assoc. Inc., New York, p. 452.
Creitz and Puckett (1954) *Am. J. clin. Path.* **24,** 1318-1323.
Falk *et al.* (1939) *J. Bact.* **37,** 121-131.
Hayden (1923) *Arch. Internal Med.* **32,** 828-849.
Hitchens (1921) *J. Infectious Diseases* **29,** 390-407.
Howell (1948) *Public Health Reports* **63,** 173-178.
Newman (1950) *J Milk and Food Tech.* **13,** 226-233.
Reitzel and Kohl (1938) *J. Am. Med. Assoc.* **110,** 1095-1098.
Roseburg *et al.* (1944) *J. Infectious Diseases* **74,** 131-149.
Rosenow (1919) *J. Dental Research* **1,** 205-249.

Brain Heart Infusion Agar

Code CM375

FORMULA

Calf Brain Infusion Solids	*grams per litre*	**12.5**
Beef Heart Infusion Solids		**5.0**
Proteose Peptone (Oxoid L46)		**10.0**
Sodium chloride		**5.0**
Dextrose		**2.0**
Disodium phosphate		**2.5**
Agar No. 1 (Oxoid L11)		**10.0**

pH 7.4 (approx.)

DIRECTIONS

Suspend 47 grams in 1 litre of distilled water. Boil to dissolve the medium completely. Distribute into tubes or flasks and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Brain Heart Infusion Agar may be recommended for the cultivation of streptococci, neisseria and other fastidious organisms.

Seth (1970) described the use of Oxoid Brain Heart Infusion with agar for the isolation of *N. gonorrhoeae.* Oxoid Brain Heart Infusion Agar was designed to be equivalent in performance. The addition of 10% defibrinated horse blood plus vancomycin 3.0 μg/ml, colistin methane sulphonate 7.5 μg/ml, nystatin 12.5 units/ml and trimethoprim lactate 8.0 μg/ml produced a specific medium which prevented the growth of Proteus species without significantly affecting *N. gonorhoeæ.*

The addition of blood and antibiotics also makes Brain Heart Infusion Agar suitable for the isolation of the tissue phase of *Histoplasma capsulatum* and other pathogenic fungi, including *Coccidioides immitis* (Howell 1948; Creitz and Puckett 1954).

REFERENCES

Creitz, J. R. and Puckett, T. F. (1954) 'Method for cultural identification of *Coccidioides immitis*' *Amer. J. clin. Path.* **24,** 1318–1323.
Howell, E. (1948) 'Efficiency of methods for the isolation of *Histoplasma capsulatum*' *Public Health Reports* **63,** 173–178.
Seth, A. (1970) 'Use of trimethoprim to prevent overgrowth by Proteus in the cultivation of *N. gonorrhoeae*' *Brit. J. veuer. Dis.* **46,** 201–202.

Brewer Thioglycollate Medium

See page 262.

Brilliant Green Agar

Code CM263

FORMULA

Proteose Peptone (Oxoid L46)	*grams per litre*	10.0
Yeast Extract (Oxoid L20)		3.0
Lactose		10.0
Sucrose		10.0
Sodium chloride		5.0
Phenol red		0.08
Brilliant green		0.0125
Agar No. 3 (Oxoid L13)		12.0

pH 6.9 (approx.)

DIRECTIONS

Suspend 50 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A highly selective plating medium, based on that of Kauffmann (1935), for the isolation and identification of salmonellae from faeces and other pathological material, and from food and dairy products. This medium was not designed for the isolation of *Salmonella typhosa* or *Shigella* species and where these may be encountered, Brilliant Green Agar should be used in parallel with other selective plating media such as Desoxycholate Citrate Agar (Hynes) CM227, SS Agar CM99, and Bismuth Sulphite Agar CM201, etc. Brilliant Green Agar may be used with enrichment media such as Selenite Broth (Oxoid CM39) and Tetrathionate Broth (Oxoid CM29).

Brilliant Green Agar corresponds to the medium recommended by the American Public Health Association (1958) for the examination of food for salmonellae, and by the APHA (1960) for the examination of dairy products for salmonellae.

COLONIAL CHARACTERISTICS

Lactose/sucrose fermenters—almost completely inhibited, occasional strains produce yellow-green colonies surrounded by a yellow-green zone.
Salmonellae—pink colonies surrounded by bright red medium.
Proteus species—some strains produce red colonies.

TECHNIQUE

Examination of faeces, or similar material, for salmonellae:

1. Heavily inoculate a Brilliant Green Agar plate. At the same time, inoculate other plating media and tubes of Selenite Broth and Tetrathionate Broth.

2. Incubate the Brilliant Green Agar plate for 18–24 hours at 37° C.

3. Examine the plates and identify suspect colonies using differential media or serological methods.

4. If no non-lactose fermenters are observed on the primary plate cultures, inoculate Brilliant Green Agar and other plates with the enrichment cultures—then proceed as in paragraph **3.**

Examination of food for salmonellae (detection and isolation):

1. Add 20 grams of homogenized sample to 80 ml of Selenite Broth.

2. Incubate for 16–24 hours at 37° C.

3. Subculture on to plates of Brilliant Green Agar and Bismuth Sulphite Agar (Oxoid CM201).

4. Incubate the Brilliant Green Agar plate for 24 hours at 37° C. Incubate the Bismuth Sulphite Agar plate for up to 48 hours at 37° C.

5. Examine the plates for colonies with *Salmonella* characteristics and identify by the usual cultural or serological methods.

Examination of food for salmonellae (enumeration):
This is carried out by adding equal volumes of decimal dilutions of the homogenized sample to tubes of double-strength Selenite Broth. After incubation, a loopful from each tube is plated on Bismuth Sulphite Agar and Brilliant Green Agar. Colonies with *Salmonella* characteristics are identified and the most probable number of salmonellae per gram of sample is calculated from the three highest sample dilutions which yield salmonellae on subculture. Reference, APHA (1958).

Examination of dairy products for salmonellae:
Milk and liquid milk products, dried milk, cheese, eggs and egg products—Brilliant Green Agar is employed, with and without an enrichment phase, in conjunction with other selective media for enteric bacteria. Reference APHA (1960).

REFERENCES

American Public Health Association (1958) 'Recommended Methods for the Microbiological Examination of Foods' APHA Inc., New York, pp. 156–159, 169.
American Public Health Association (1960) 'Standard Methods for the Examination of Dairy Products' 11th ed., APHA Inc., New York, pp. 152–154, 406.
Kauffmann, F. (1935) 'Weitere Erfahrungen mit den Kombinierten Anreicherungsuerfahren fur *Salmonella* bacillen *Zeit. f. Hyg.* **117**, 26.

Brilliant Green Agar (MODIFIED)

Code CM329

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	5.0
Bacteriological Peptone (Oxoid L34)		10.0
Yeast Extract Powder (Oxoid L21)		3.0
Disodium hydrogen phosphate		1.0
Sodium dihydrogen phosphate		0.6
Lactose		10.0
Sucrose		10.0
Phenol red		0.09
Brilliant green		0.0047
Agar No. 1 (Oxoid L11)		12.0

pH 6.9 (approx.)

DIRECTIONS

Suspend 52 grams in 1 litre of distilled water. Heat gently with occasional agitation and bring just to the boil to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 50° C, mix well and pour plates.

DESCRIPTION

Brilliant Green Agar (Modified) was developed from a formula supplied by the Rijks Instituut voor de Volksgezondheid (National Institute for Public Health), Utrecht.

The work carried out at Utrecht has shown that the Oxoid medium closely resembles that produced in their own laboratories. The following papers have been published from the Institute on the subject of salmonella isolation, using R.I.V. Brilliant Green Agar: (Guinée & Kampelmacher, 1962. Guinée, Kampelmacher & Hoejenbos-Spithout, 1965; Van Schothorst & Kampelmacher, 1967; Kampelmacher, 1967; Edel & Kampelmacher, 1968).

The advantages claimed for the medium are the greater inhibition of Escherichia coli and Proteus species than other formulations: the restriction of growth of Pseudomonas species, whose colonies may resemble salmonellae on Brilliant Green Agar and cause confusion or much extra work to confirm their identity: the absence of inhibitory properties towards small numbers of salmonellae, as discussed by Read and Reyes (1968).

Oxoid produced dehydrated Brilliant Green Agar in a two part presentation for use in a large scale investigation, between nine laboratories in eight different countries (Edel & Kampelmacher, 1969). The single pack version of this medium, here presented, represents an improvement both in quality and in convenience of use over the original twin pack medium.

COLONIAL CHARACTERISTICS

Salmonellae—red colonies surrounded by bright red medium.

Lactose/Sucrose fermenters—inhibited to a certain extent, but producing yellow green colonies when growth is evident.

Proteus—almost completely inhibited, those colonies that grow produce red colonies without swarming.

Pseudomonas—inhibited growth of small, crenated red colonies.

TECHNIQUE

An outline of the method used in the above mentioned trial is as follows:

'Oxoid' Tetrathionate Broth was modified by the addition of 4.75 grams Desiccated Ox Bile and 0.0095 grams Brilliant Green per litre to prepare the Muller-Kauffmann* formula, as a liquid enrichment medium for the salmonellae.

One part of sample to twenty parts of enrichment broth was prepared and the flasks of broth placed immediately after inoculation into a 45° C water-bath for 15 minutes. After this time, the flasks were transferred to a 43° C incubator.

Subcultures were made from the enrichment broth after 18 hours and 48 hours incubation.

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* Oxoid now offer a Muller-Kauffmann Broth Base, CM343.

A single loopful of broth was used to streak-inoculate either two Brilliant Green Agar plates (9 cm diameter), without re-charging the loop between plates, or one large plate (14 cm).

The Brilliant Green Agar plates were incubated at 37° C for 18–24 hours.

Red colonies, resembling salmonellae, were picked off the plates and subcultured into Lysine Decarboxylase Broth and Triple Sugar Iron Agar. Both media were incubated at 37° C for 18–24 hours.

If the reactions of the Lysine Decarboxylase Broth and Triple Sugar Iron Agar were positive, slide agglutination tests were carried out on the culture growing on the surface of the Triple Sugar Iron Agar.

REFERENCES

Edel, W. and Kampelmacher, E H. (1968) 'Comparative studies on salmonella-isolation in eight European laboratories' *Bull. Wld. Hlth. Org.* **39**, 487–491.

Edel, W. and Kampelmacher, E. H. (1969) 'Salmonella isolation in nine European laboratories using a standardized technique' *Bull. Wld. Hlth. Org.* **41**, 297–306.

Guinée, P. A. M. and Kampelmacher, E. H. (1962) 'Influence of variations of the enrichment method for the detection of salmonella' *Antonie van Leeuwenhoek* **28**, 417–427.

Guinée, P. A. M., Kampelmacher, E. H. and Henriette H. M. Hoejenbos-Spithout (1965) 'Further studies on the influence of variations in the enrichment for the detection of salmonella' *Antonie van Leeuwenhoek* **31**, 1–10.

Kampelmachet, E. H. (1967) 'Comparative studies into the isolation of salmonella from minced meat in five laboratories. Report from a working group' *Zentbl. Bakt. I. Orig.* **201**, 100–111.

Read, R. B. and Reyes, A. L. (1968) 'Variation in Plating efficiency of salmonellae on eight lots of Brilliant Green Agar' *Appl. Microbiol.* **16**, 746–748.

Van Schothorst, M. and Kampelmacher, E. H. (1967) 'Salmonella in meat Imported from South American countries *J. Hyg. Camb.* **65**, 321–325.

Brilliant Green Bile (2 %) Broth

Code—Powder CM31
Tablets CM32

FORMULA

Peptone (Oxoid L37) . . . . *grams per litre*	**10.0**
Lactose	**10.0**
Ox Bile (purified)	**20.0**
Brilliant green	**0.0133**

pH 7.4 (approx.)

DIRECTIONS

Powder: Add 40 g to 1 litre of distilled water. Mix well, distribute into containers fitted with Durham's tubes and sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water and insert a Durham's tube. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This medium may be used to detect or confirm the presence of members of the *coli-aerogenes* group of organisms. Brilliant green bile broth works on the same principle as MacConkey broth but Mackenzie *et al.* (1948) found that the brilliant green content effectively suppressed anaerobic lactose-fermenters, such as *Clostridium perfringens* (*Cl. welchii*) which give rise to false positive reactions at 44° C. Using *Cl. perfringens* as the indicator organism, the brilliant green prepared for the Oxoid medium is titrated to produce optimum suppression of spore-forming organisms and maximum recovery of *Escherichia coli.* Brilliant green bile broth is recommended in 'Standard Methods for the examination of Water and Wastewater' for confirmation of the presumptive test for coliforms; whilst in 'Standard Methods for the Examination of Dairy Products', the medium is recommended for use in the actual presumptive test for coliform organisms.

Labots and Galesloot (1960) found that Oxoid Brilliant Green Bile Broth incubated at 30° C was suitable for the detection of coliforms in milk.

TECHNIQUE

In the version of Mackenzie's method recommended by Windle Taylor (1958), presumptive positive MacConkey broth tubes are subcultured into brilliant green bile broth and peptone water. Both tubes are incubated at 44° C and after 6 hours and 24 hours the peptone water is examined for indole whilst the brilliant green bile broth is examined for turbidity and gas production—the latter is indicated by effervescence when the tube of medium is tapped, and by a gas bubble inside the Durham tube (i.e. a positive reaction). Certain coliform organisms besides *E. coli* I will give a positive result with brilliant green bile broth, so that the indole test is necessary in order to differentiate them: Brilliant green positive and indole positive indicates *E. coli* I; brilliant green positive and indole negative indicates Irregular types II or VI. By virtue of its high tryptophane content, Tryptone Water (Code CM87) is superior to Peptone Water (Code CM9) for the cultivation of organisms which are to be tested for indole production.

In American procedures, tubes of inoculated brilliant green bile broth are incubated for 48 hours at 35° C for the presumptive test, the inocula consisting of dilutions of the original sample. When used as a confirmatory medium, the broth is inoculated from primary fermentation tubes (lactose broth or lauryl tryptose broth) showing gas after 24 or 48 hours.

In Australian procedures, Lightbody (1963) found that brilliant green bile broth or MacConkey broth is best incubated at 32° C for the presumptive coliform test on dairy products.

REFERENCES

American Public Health Association (1967) Standard Methods for the Examination of Dairy Products' 12th ed., APHA Inc., New York, pp.54, 57, 59-64.

American Public Health Association, American Water Works Association, Water Pollution control Federation (1965) 'Standard Methods for the Examination of Water, Wastewater' 12th ed., APHA Inc., New York, pp. 596-597.

Labots, H. and Galesloot, Th. E. (1960) 'The Suitability of the Powdered Brilliant Green Bile Medium (BGB) made by Oxo Ltd. for the detection of Coliform Bacteria in Milk' *Rapp. Ned. Inst. Zuivelonderz.* **25.**

Lightbody, Lorna G. (1963) 'Presumptive Coliform Tests in Liquid Media at 32° and 37° C' *Aust. J. Dairy Tech.* **18**(4), 202–203.

Mackenzie, E. F. W., Windle Taylor, E., Gilbert, W. E. (1948) 'Recent Experiences in the Rapid Identification of Bacterium coli Type I' *J. gen. Microbiol.* **2,** 197–204.

Windle Taylor, E. (1958) 'The Examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London, pp. 439–440.

Brucella Medium Base

Serum Dextrose Agar Base
Code CM169

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10.0**
'Lab-Lemco' Powder (Oxoid L29)		**5.0**
Dextrose		**10.0**
Sodium chloride		**5.0**
Agar No. 3 (Oxoid L13)		**15.0**

pH 7.5 (approx.)

DIRECTIONS

Suspend 45 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C and add 5% of inactivated Horse Serum (i.e. serum held at 56° C for 30 minutes). Mix well before pouring.

The following antibiotics may be added to the above serum-dextrose agar:

Bacitracin	25,000 units per litre
Polymyxin B	600 µg per litre
Actidione	100 mg per litre

N.B. It is important to ensure thorough mixing in order to obtain uniform results.

DESCRIPTION

Oxoid Brucella Medium Base may be used to prepare the serum-dextrose antibiotic medium described by Jones and Brinley Morgan (1958) for the cultivation and isolation of *Brucella,* including fastidious types. Brucella Medium with antibiotics has advantages over the media described by Kuzdas and Morse (1953) and Renoux (1954) in that it will support growth of fastidious types and it is more effective as a selective medium. During investigations, Jones and Brinley Morgan showed that serum-dextrose agar with antibiotics gave excellent growth of all *Brucella* strains and permitted better growth of *Brucella abortus* type II—a strain which had been difficult or impossible to cultivate.

TECHNIQUE

The addition of dyes (i.e. malachite green and gentian violet) as selective agents, is not recommended, as it may result in poor growth of many *Brucella* strains. Where a non-selective medium is required, the Oxoid base may be employed with the addition of serum only (i.e. without antibiotics), and for subsequent differentiation between strains of *Brucella* this medium is recommended for use in conjunction with the Cruickshank dyestrip method (1948):

1. Impregnate filter paper strips with 1:200 Basic Fuchsin or 1:600 Thionin. Dry.

2. Place the strips parallel on the surface of the serum dextrose agar and then cover with a thin layer of the same medium. Then allow the medium to solidify.

3. Make stroke inoculations of the *Brucella* strains to be tested, at right angles to the strips.

4. Incubate in 10% carbon dioxide for 2–3 days at 37° C.

5. Examine. Resistant strains grow right across the strip, but sensitive strains show inhibition of growth up to 10 mm from the strip. Typical growth patterns are then as follows:

	Basic Fuchsin 1:200	Thionin 1:600
Brucella abortus	growth	no growth
Brucella melitensis	growth	growth
Brucella suis	no growth	growth

However, there are exceptions to the above and it is therefore advisable to base identification on many characteristics. For an account of *Brucella* taxonomy, see Stableforth and Jones (1963).

REFERENCES

Cruickshank, J. C. (1948) 'A Simple Method for Testing Dye Sensitivity of *Brucella* Species. *J. Path. Bact.* **60**, 328–329.

Jones, Lois M. and Brinley Morgan, W. J. (1958) 'A Preliminary Report on a Selective Medium for the Culture of *Brucella*, including Fastidious Types' *Bull. Wld Hlth Org.* **19**(1) 200–203.

'Kuzdas, C. D. ahd Morse, E. V. (1953) 'A Selective Medium for the Isolation of Bruceliae from Contaminated Materials' *J. Bact.* **66**(4), 502–504.

Renoux, G. (1954) 'A Selective Medium for the Isolation of *Brucella melitensis*' *Ann. Inst. Pasteur* **87**(3), 325–333.

Stableforth, A. W. and Jones, Lois M. (1963) Report of the Subcommittee on Taxonomy of the Genus *Brucella*' *Internat. Bull. Bact. Nomen. Taxon.* **13**(3), 145–158.

Buffered Pancreatin Tablets

Code BR1

FORMULA

Pancreatin	*grams per litre*	**10.0**
Sodium chloride		**8.5**
Phosphate buffer		**1.28**

pH 7.5

DIRECTIONS

Using aseptic technique, place 1 tablet in 50 ml sterile distilled water at 37° C. Place the container in a 37° C water bath or incubator and dissolve the tablet by constant agitation. After the tablet has completely dissolved, cool the solution to room temperature if it is not to be used immediately.

DESCRIPTION

Radiation sterilized tablets which dissolve in water to give a clear sterile solution for sputum liquefaction, prior to microbiological or cytological investigation. Gamma irradiation sterilization does not affect the enzyme activity of this preparation.

Following earlier work by Rowan and Rice (1952), Rice and Rowan (1953), and Rawlins (1953), it was suggested by Kohn (Personal Communication) that buffered pancreatin in tablet form might have many advantages. Consequently the tablets were developed in the Oxoid Laboratories to provide a convenient source of sputum liquefying solution. Most pancreatin solutions are turbid and contain fibrous particulate matter. The solution made from Oxoid Buffered Pancreatin Tablets is free from insoluble matter, so that there is no interference with the examination of delicate cell structures, etc.

May (1952, 1953) showed that bacteria are often unevenly distributed in the sputum of patients suffering from chronic bronchitis, and that single cultures may fail to reveal all the bacterial species present. Rawlins' (1955a) enzymic method of sputum liquefaction produced a uniform suspension, without interfering with the bacterial flora. This made it possible to give a satisfactory estimate of the proportions of each bacterial species present in the specimen. Rawlins (1955b) also showed that pancreatin is not cytolytic and that the liquefied sputum could be used for the determination of the number of pus cells; counts of the total number of cells should be made on the day the sputum is liquefied, but stained films should always be prepared immediately after liquefaction. Ortiz Masllorens and Alés Reinlein (1961) employed Rawlins' (1955a) method for several years. On the basis of a comparative study of 210 sputa examined by Rawlins' method and also by conventional methods, they found that the pancreatin method was better in 57.5% of cases, equally valuable in 38.7%, and worse in only 3.8%. They found that the pancreatin method did not impair bacterial viability.

Delicate organisms, such as *Streptococcus pneumoniae* and *Haemophilus influenzae* are not affected by the pancreatin treatment.

Rice and Rowan (1953) described a technique using pancreatin as a concentrating agent for tuberculous material. They showed that this technique, with subsequent cultivation on Löwenstein-Jensen medium, yielded more rapid and profuse growth of tubercle bacilli than Petroff's method applied to the same material.

Stewart and Norris (1959) employed Oxoid Buffered Pancreatin solution for the demonstration of malignant cells in sputum. They added an equal volume of solution to sputum in a sterile container, incubated for 90 minutes at 37° C., centrifuged the specimen and then made two smears of the deposit—these were fixed in Schaudinn fluid and stained with haemalum and eosin. The concentration method was strikingly successful, showing much greater sensitivity than the direct method.

To summarize, the advantages of Buffered Pancreatin Tablets are:

(i) Each tablet provides a source of sputum liquefying solution.

(ii) The prepared solution is sterile and free from insoluble matter.

(iii) There is no effect on the bacterial flora—delicate pathogens such as *D. pneumoniae* and *H. influenzae* may be recovered.

(iv) Culture is made from a representative sample containing a selection of all the organisms present in the specimen.

(v) The solution is non-cytolytic to living cells. Pus cell, total cell, and differential cell counts can be made and malignant cells may be demonstrated.

(vi) Average sputa are liquefied in 90 minutes or less.

(vii) The method may be used for the concentration of *Mycobacterium tuberculosis* prior to micro-culture or membrane filtration.

TECHNIQUE

The procedure adopted for the routine liquefaction of sputum, based on Rawlins' papers, is as follows:

1. The sputum is expectorated into a sterile Universal container or other wide mouthed screw-capped bottle.

2. Add approximately 5 times the volume of 0.85% saline and agitate to free the sputum from adherent saliva. Remove the saline with a sterile Pasteur pipette.

3. To the washed sputum add an equal volume of the solution prepared from a Buffered Pancreatin Tablet.

4. Shake the resultant mixture well. The rate at which liquefaction occurs is partially dependent on the efficiency of the mixing. Five minutes shaking is desirable, but efficient shaking can only be achieved if the volume of mixture is less than two-thirds of the volume of the container.

5. Place the mixture in a 37° C water bath and incubate, with periodic shaking, until liquefaction is complete. The rapidity with which air bubbles rise to the surface after shaking provides a convenient indication of the progress of liquefaction. Most sputa are rendered completely liquid in 90 minutes or less. Some thick tenacious sputa may require longer incubation, or the addition of more enzyme solution and more vigorous shaking.

6. Mix well, and inoculate a suitable culture medium. For the total cell count, place a drop of the liquefied sputum in a haemo-cytometer and enumerate the cells in the usual manner. For the differential cell count, fix a dried smear in methyl alcohol and stain with haematoxylin and eosin or Leishman stain.

REFERENCES

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Stewart, J. and Norris, P. J. (1959) 'Letters to the Editor Demonstration of Malignant Cells in Sputum' *Lancet* 19/12/59, 1149.

Buffered Yeast Agar

Code—Powder CM153
Tablets CM154

FORMULA

	grams per litre
Yeast Extract (Oxoid L20)	**5.0**
Dextrose	**20.0**
Ammonium sulphate	**0.72**
Ammonium dihydrogen phosphate	**0.26**
Agar No. 3 (Oxoid L13)	**12.0**

pH 5.5 (approx.)

DIRECTIONS

Powder: Suspend 38 g in 1 litre of distilled water. Bring to the boil to dissolve. Sterilize by autoclaving at 115° C for 20 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 115° C for 20 minutes.

DESCRIPTION

Oxoid Buffered Yeast Agar is a modification of the yeast-salt medium described by Davis (1931). The medium is recommended as a semi-synthetic medium for the cultivation of yeasts and moulds, and for the control of bottle washing operations in the 'soft' drinks and allied industries.

Davis (1931) showed that the reaction of the medium may be readily adjusted to the required pH value by the addition of citric acid. The following table gives the amount of citric or lactic acid to be added to the medium after sterilization to adjust the reaction of the medium to various pH values (the pH values were measured at room temperature). The sterile molten medium should be cooled to approximately 50° C before the addition of acid.

Volume of acid to 100 ml of Oxoid Buffered Yeast Agar:

pH	Solution of Citric Acid monohydrate 1% w/v ml	Solution of Lactic Acid 10%* ml
4.75	1.26	0.125
4.50	2.24	0.20
4.25	3.92	0.30
4.00	6.16	0.45
3.75	9.52	0.70
3.50	14.56	1.17

* Available sterile, in ampoules, as Oxoid Lactic Acid 10% SR21.

A practical method for assessing the efficiency of bottle cleaning operations is that described by Bunker (1952, 1956). Essentially it consists in converting the bottle under test into a 'roll tube' culture by coating it internally with a suitable agar medium. When the agar has set, the bottle is incubated and the colonies counted and examined. This technique gives better results than rinsing the bottle and subsequently plating the rinsings. Oxoid Buffered

Yeast Agar can be recommended for the examination of bottles for mould or yeast contamination using the Bunker roll bottle method. For this purpose the agar concentration must be increased by the addition of 1% w/v of agar to the medium before sterilization. This is conveniently added in the form of Oxoid Agar Tablets (Code CM49): 8 tablets should be added to each 100 ml of medium, sufficient for a quart bottle. Incubate at room temperature and examine daily.

Roll bottle cultures for anaerobic lactobacilli, etc., should be incubated anaerobically.

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Davis, J. G. (1931) 'Standardization of Media in the Acid Ranges with Special Reference to. the Use of Citric Acid and Buffer Mixtures for Yeast and Mold Media' *J. Dairy Res.* **3**, 133–141.
Bunker, H. J. (1952) 'Biological Control in Brewing and Bottling' *Lab. Prac.* **1**(8), 354–359
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Casein Hydrolysate

See page 210

Charcoal Agar

Code CM119

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**10.0**
Peptone (Oxoid L37)		**10.0**
Starch		**10.0**
Charcoal-Bacteriological (Oxoid L9)		**4.0**
Sodium chloride		**5.0**
Nicotinic Acid		**0.001**
Agar No. 3 (Oxoid L13)		**12.0**

pH 7.4 (approx.)

DIRECTIONS

Suspend 51 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C, add 10% of defibrinated blood and mix gently. Render selective for *Bordetella pertussis* by adding 0.3 unit penicillin and 2μg 4:4'-diamidinodiphenylamine dihydrochloride (M & B 938)* per ml. For *Haemophilus influenzae*, omit selective agents and convert to 'chocolate' agar.

DESCRIPTION

This formula was developed in the Oxoid Laboratories and provides a simple selective medium for *Bordetella pertussis* or for the cultivation of *Haemophilus influenzae*, etc.

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* Available in vials of 1 gram from May and Baker Ltd., Dagenham.

Ensminger *et al.* (1953) employed a charcoal medium for the production of *Bordetella pertussis* vaccines, and found that the medium could replace Bordet-Gengou medium for this purpose. Mishulow *et al.* (1953) also employed a charcoal medium for *Bordetella pertussis* cultivation and found that the medium was an efficient substitute for Bordet-Gengou. Brumfitt (1959) found that growth of *B. pertussis* on charcoal 'chocolate' agar was comparable to that on Bordet-Gengou.

Proom (1955) showed that nicotinic acid was an essential nutrient for *B. pertussis, B. parapertussis* and *B. bronchiseptica.*

Bradford and Slavin (1940) concluded that poor results with the cultivation of post-nasal swabs for *B. pertussis* were due to overgrowth by rival organisms rather than to the absence of *B. pertussis.* Lacey (1954) was able to confirm this—he employed a selective medium containing M & B 938 which suppressed rival organisms and consequently yielded more positive cultures for *B. pertussis.*

TECHNIQUE

For the isolation of *B. pertussis*—Charcoal Agar is employed with added M & B 938, penicillin and blood. The addition of only 10% of blood is required (as opposed to 15–50% with Bordet-Gengou medium) although some *pertussis* strains will grow almost as well in its complete absence. Penicillin and M & B 938 almost entirely suppress growth of the normal nasopharyngeal flora, including *H. influenzae.* After inoculation with prenasal swab or other material the plate is incubated for up to 3 days at 37° C. *B. pertussis* is in the 'smooth phase' when grown on this medium and produces small, pale, drop-like, glistening colonies which may be identified by microscopy and agglutination.

H. influenzae is cultivated on the medium containing 10% of 'chocolated' blood but no M & B 938 or penicillin. The inoculated plate is incubated for 2 to 3 days at 37° C. The colonies are usually small, transparent and droplet-like, but some transformation to the 'rough' type colony may occur. Species differentiation is performed by examination for the need for X and V growth factors, on Blood Agar Base (Code CM55). See page 288.

REFERENCES

Bradford, W. L. and Slavin, B. (1940) Nasopharyngeal Cultures in Pertussis' *Proc. Soc. exp. Biol.* **43**, 590.
Brumfitt, W. (1959) 'Some Growth Requirements of *Haemophilus influenzae* and *Haemophilus pertussis*' *J. Path. Bact.* **77**(1), 95–100.
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Proom, H. (1955) 'The Minimal Nutritional Requirements of Organisms of the Genus *Bordetella* López' *J. gen. Microbiol.* **12**(1), 63–75.

Charcoal-Bacteriological

Code L9

A specially purified activated carbon, of value for incorporation in culture media, and for other purposes where its ability to remove bactericidal and bacteriostatic substances greatly facilitates the cultivation of fastidious organisms.

The use of charcoal for bacteriological purposes has been described many times in technical literature:

(i) Hirsch (1954a) employed charcoal media for the isolation of tubercle bacilli. It was found that the addition of charcoal to a modified Dubos medium (containing no albumin) permitted growth of small inocula of the organism, and that charcoal was able to detoxify oleic acid or Tween 80 added to the medium. The charcoal agar medium evolved gave larger colonies than Löwenstein-Jensen or oleic-acid-albumin media. It was also found (Hirsch, 1954b) that dilute suspensions of activated charcoal, in water or phosphate buffer, served as well as bovine plasma albumin as a protective diluent for tubercle bacilli but had advantages (over albumin water) of thermostability, economy, availability, and freedom from the ability to sensitize recipient animals.

(ii) Mishulow *et al.* (1953) cultivated *Bordetella pertussis* (*Haemophilus pertussis*) on a charcoal medium. This medium was an efficient substitute for Bordet-Gengou medium for the preparation of *pertussis* vaccines, which were shown to be more potent than the standard reference vaccine.

(iii) Ensminger *et al.* (1953) used an activated charcoal medium for the production of *Bordetella pertussis* vaccines. The authors found that the medium could replace Bordet-Gengou medium, and that charcoal was the most effective adsorbent investigated.

(iv) Stuart *et al.* (1954) employed charcoal swabs for the transport of material to be subsequently cultivated for *Neisseria gonorrhoeae.* The swabs were impregnated with charcoal suspension, then dried and sterilized before use. The charcoal neutralized inhibitory substances contained in the agar of the transport medium, leading to increased recovery of gonococci. It was noted that the transport method was applicable to organisms other than *Neisseria,* including *Trichomonas, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes* and *Corynebacterium diphtheriae.*

(v) Cooper (1957) investigated the extension of Stuart's method to the transport of swabs of clinical material containing upper respiratory tract or enteric pathogens. He suggested that not only did the charcoal (in the swab) remove the toxic fatty acids present in some agars, but that it might also absorb toxic metabolites produced by the organisms themselves.

Charcoal Agar CM 119, containing Charcoal-Bacteriological, is used as a basal medium for differentiation and cultivation of *Haemophilus* and *Bordetella* species, etc.

REFERENCES

Cooper, G. N. (1957) 'The Prolonged Survival of Upper Respiratory Tract and Intestinal Pathogens on Swabs' *J. clin. Path.* **10**(3), 226–230.
Ensminger, P. W., Culbertson, C. G. and Powell, H. M. (1953) 'Antigenic *Hemophilus pertussis* Vaccines Grown on Charcoal Agar' *J. Infect. Dis.* **93**(3), 266–268.
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Charcoal Gelatin Discs

Code BR10

(A Rapid and Convenient Test for Gelatin Liquefaction.)

Oxoid Charcoal Gelatin Discs are sterile pellets prepared from formalin-denatured gelatin and finely powdered charcoal, as described by Kohn (1953a and b) for a new method of demonstrating gelatin liquefaction. The gelatin charcoal is used only as a substrate for testing for gelatinase activity and not as a nutrient medium. The disc material contains a large number of fine particles of charcoal bound together by a comparatively small amount of gelatin. Consequently, even a small degree of liquefaction will release sufficient charcoal particles to provide an indication of gelatinase activity.

The Advantages of the Method:

(i) Results can often be obtained in a matter of hours.

(ii) The medium may be incubated at any desired temperature since denatured gelatin does not melt.

(iii) Charcoal Gelatin Discs can be added to any culture medium whether liquid, semi-solid or solid. This eliminates the need for a separate gelatin medium.

(iv) They can be added to established cultures, and if a gelatin liquefier is present, the gelatinase, which will already have been formed in the medium, will rapidly be detected.

(v) It compares very favourably in cost with existing methods of detecting gelatinase activity—in all cases it compares favourably with nutrient gelatin (see the table).

To Test for Gelatinase Activity:

Charcoal Gelatin Discs can readily be removed from the bottle by touching an exposed disc with a hot wire, when it will adhere. It can then be transferred to a culture without contamination. Add a disc to the inoculated medium or to the established culture and incubate in the normal way. It is preferable to use an agar slope and place the disc near the bottom of the tube, taking care that there is some condensation water present. Plate cultures, in general, are not suitable.

Liquefaction is first shown by the appearance of free charcoal particles settling to the bottom of the medium and, later, the disc completely disintegrates with the formation of a clearly visible black cloud. Mechanical actors such as shaking do not produce false positives.

Speed of Action:

Green & Larks (1955) described a rapid test employing 1 ml volumes of peptone water in small test tubes. A charcoal gelatin disc, prepared according to Kohn's directions, was added after the tubes of medium had been warmed to 37° C for 15 minutes. The tubes were heavily inoculated from young nutrient agar slope cultures, incubated in a water bath, and examined at frequent intervals for evidence of liquefaction. Fifteen cultures of gelatin liquefiers were tested by this method, by Kohn's original method and by the conventional gelatin tube method. Controls of non-liquefiers showed no liquefaction. The table shows time required for detectable liquefaction and demonstrates clearly the rapidity of the test.

Organism	Conventional Gelatin Tube Method	Kohn's Original Method	Greene and Larks Micro Method
Bacillus alvei	6 days	3 days	4 hours
Micrococcus citreus . .	7 days	1 day	2 hours
Proteus vulgaris . . .	2 days	1 day	2 hours
Pseudomonas fluorescens .	3 days	1 day	3 hours
Streptococcus liquefaciens .	2 days	1 day	4 hours
Vibrio metschnikovii . .	8 days	3 days	6 hours

More recently, Saccani (1956), compared Kohn's method with the normal gelatin tube method, incubated at 18° C and 37° C, and found that the proteolytic activity of the test bacteria developed more rapidly using Kohn's technique. Lautrop (1956a) described a modified method for obtaining very rapid results, as follows: A concentrated suspension of the organisms is prepared by emulsifying all the growth from an 8 cm Petri dish culture in 3 to 4 ml of suspension medium. The latter should consist of 0.01 M calcium chloride in physiological saline. Incubate at 37° C and examine at frequent intervals up to three days. Phosphate buffers are not suitable for routine tests as Lautrop showed that calcium ions are essential for gelatinase production in some genera and that calcium-precipitating ions, such as phosphates and oxalates, seem to inhibit gelatinase formation in some strains. In his observations on gelatin-liquefying strains of the *Salmonella* group, Lautrop (1956b) demonstrated the existence of two different types of gelatin-liquefying enzymes using a modification of Kohn's test. The rapid liquefiers in gelatin stab cultures, i.e. *S. abortus bovis, S. schleissheim* and *S. texas,* were the only types that contained a special type of gelatinase requiring the presence of calcium ions for full activity. One proposed procedure is as follows: Perform simultaneously the modified Kohn's test in two different ways: (a) as for the demonstration of normal gelatinase activity, and (b) with the growth from a slope, grown at 22° C, suspended in saline containing 0.01 M calcium chloride. If the test is performed in this way, using a dense suspension, the calcium requiring enzyme can be demonstrated in less than 24 hours.

REFERENCES

Greene, R. A. and Larks, Golda G. (1955) 'A Quick Method for the Detection of Gelatin Liquefying Bacteria' *J. Bact.* **69**, 224.
Kohn, J. (1953a) 'A Preliminary Report of a New Gelatin Liquefaction Method' *J. clin. Path.* **6**, 249.
Kohn, J. (1953b) 'The Use of Denatured Gelatin for Biochemical Tests' Ibid., 250.
Lautrop, H. (1956a) 'A Modified Kohn's Test for the Demonstration of Bacterial Gelatin Liquefaction' *Acta Path. Microbiol. Scand.* **39**, 357–369.
Lautrop, H. (1956b) 'Observations on Gelatin-Liquefying Strains of the *Salmonella*-Group' Ibid., 370–374.
Saccani, C. F. (1956) 'A Rapid Method for the Demonstration of Gelatin Liquefaction *Bolletino Ist. d'Igiene Univ. Milano* **35**, 9–10.

China Blue Lactose Agar

Code CM209

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**5.0**
'Lab-Lemco' Powder (Oxoid L29)		**3.0**
Lactose		**10.0**
Sodium chloride		**5.0**
China blue		**q.s.**
Agar No. 3 (Oxoid L13)		**12.0**

pH 7.0 (approx.)

DIRECTIONS

Suspend 35 g in 1 litre of distilled water. Boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A standard, non-inhibitory solid medium for the differentiation and enumeration of bacteria in milk, proposed by the Methodenkommission für Milchwirtschaft. The china blue serves as a pH indicator, to differentiate between lactose fermenters and non-lactose fermenters, but does not suppress the growth of cocci; therefore this medium may be used for the detection of streptococci and staphylococci as well as the *coli-aerogenes* group. After 18 hours' incubation at 37° C, colony appearances are as follows:

Escherichia coli—3 to 4 mm diameter, light blue with dark blue centre.
Non-lactose fermenters—smaller, colourless.
Enterococci—approximately 0.5 mm diameter, blue.
Staphylococci—green-blue, opaque, approximately 1 mm diameter.

REFERENCES

Methodenbuch Band VI 'Chemische, physikalische und bakteriologische Untersuchungsverfahren für Milch, Milcherzeugnisse und Molkereihilfstoffe', Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. 1970.
Demeter, 'Bakteriologische Untersuchungsmethoden der Milchwirtschaft' Ulmer Verlag, Stuttgart. 1952, Seite 28.

Cholera Medium

Lauryl Sulphate Tellurite Agar

Code CM237

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10.0**
'Lab-Lemco' Powder (Oxoid L29)		**10.0**
Sucrose		**10.0**
Sodium lauryl sulphate		**0.1**
Sodium chloride		**20.0**
Sodium carbonate		**5.0**
Agar No. 1 (Oxoid L11)		**10.0**

pH 8.5 (approx.)

AMO-236

DIRECTIONS

Suspend 61.2 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Cool to 75° C and add 2 ml of Tellurite-blood solution.* (A 1% solution of potassium tellurite in defibrinated horse blood). Maintain at 75° C for a few minutes and cool to 50° C before distributing.

DESCRIPTION

Cholera Medium is a selective medium corresponding to the formula described by Felsenfeld and Watanabe (1958), for the isolation of *Vibrio comma* (*Vibrio cholerae*) and similar vibrios from specimens grossly contaminated with *Enterobacteriaceae*. Previous media have either been exceedingly difficult to prepare or else have permitted too great a growth of contaminants. Using inocula consisting of *Vibrio* species heavily contaminated with *Escherichia coli, Aerobacter aerogenes, Proteus morganii, Pseudomonas aeruginosa* and *Bacillus subtilis*—Felsenfeld and Watanabe proved that their medium was very selective after less than 48 hours' incubation. The contaminating flora only became evident after 48 hours—although, even then, *Vibrio* colonies could still be selected with ease. One hundred and fifty *Vibrio* strains (including El Tor types) were tested, and 113 strains grew on the new medium. Of the 37 strains which failed to grow, only 4 were cultivable on Aronson medium.

In all cases, *Vibrio* colonies grown on lauryl sulphate tellurite agar gave rapid positive slide agglutination tests. Thirty stools, from patients suffering with diarrhoea which did not involve vibrios, were plated on the medium and showed no growth of any organism in 24 hours.

TECHNIQUE

Streak the faeces across the surface of a Cholera Medium plate and incubate for 20 to 24 hours at 37° C. At the same time inoculate a tube of Peptone Water CM9 (adjusted to pH 8.2) with a mucus flake or loopful of the specimen (see Cruickshank, 1962), and incubate for 6 to 8 hours at 37° C.

On the Cholera Medium plate, all *V. commae* produce translucent, greyish colonies which turn blacker on further incubation, whilst the contaminant colonies are not discernible at 24 hours.

Make stained films and hanging-drop preparations from the Peptone Water culture, and examine microscopically for vibrios.

REFERENCES

Felsenfeld, O. and Watanabe, Y. (1958) 'An Alkaline Tellurite Lauryl Sulphate-Salt Plate for the Isolation of *Vibrio comma*' *U.S. Armed Forces Med. J.* **9**(7), 975–982.

Cruickshank, R. (1962) 'Mackie and McCartney's Handbook of Bacteriology' Livingstone Ltd., London, pp. 629–630.

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* Prepared by adding the contents of one 2 ml ampoule of sterile Potassium Tellurite 3.5% Code SR30 to 5ml of defibrinated ox blood.

Clausen Medium

DITHIONITE-THIOGLYCOLLATE (HS-T) BROTH

Code CM353

FORMULA

Tryptone (Oxoid L42) *grams per litre*	**15.0**
Yeast Extract (Oxoid L21)	**6.0**
Soya Peptone (Oxoid L44)	**3.0**
Dextrose	**6.0**
Sodium chloride	**2.5**
Dipotassium hydrogen phosphate	**2.0**
Sodium citrate	**1.0**
l-Cystine	**0.5**
l-Asparagine	**1.25**
Sodium dithionite	**0.4**
Sodium thioglycollate	**0.5**
Lecithin	**0.3**
Magnesium sulphate $.7H_2O$	**0.4**
Calcium chloride $.2H_2O$	**0.004**
Cobalt sulphate $.7H_2O$	**0.001**
Cupric sulphate $.5H_2O$	**0.001**
Ferrous sulphate $.7H_2O$	**0.001**
Zinc sulphate $.7H_2O$	**0.001**
Manganese chloride $.4H_2O$	**0.002**
Resazurin	**0.001**
Agar No. 1 (Oxoid L11)	**0.75**

pH 7.1 (approx.)

DIRECTIONS

Suspend 40 g in a solution composed of Tween 80 (polyethylene sorbitan mono-oleate) 3 g : glycerol 5 g and distilled water 1 litre. Bring to the boil to dissolve completely.

Distribute into tubes or bottles and sterilize by autoclaving at 121° C for 15 minutes.

THE MEDIUM MUST NOT BE RE-STERILIZED.

DESCRIPTION

Dithionite-thioglycollate (HS-T) Broth was developed by Clausen in Oslo University and has been recommended for sterility testing by the Nordic Pharmacopoeia Board. The problems of sterility testing by selecting random samples is recognised by the Board and they refer to the process as Microbial-Contamination Test. The standard microbial-contamination test is designed solely to establish that the number of non-sterile units, if any, in a batch is below a certain level.

The following description of the Standard Microbial-Contamination Test has been abridged from the detailed description to be published as an addendum in the Nordiska Farmakopenämnden.

The tests must be performed with all precautions taken to prevent laboratory contamination occurring more than once at the most in every 100 tests. The use of laminar air-flow cabinets is recommended. Tests are to be made by qualified and experienced staff and the efficiency of the methods used must be checked at regular intervals.

A random sample of sufficient quantity to be representative of the whole bulk, should be examined.

Two methods of detecting non-sterile units may be used in the microbial-contamination test.

METHOD I (MEMBRANE FILTER METHOD)

The test substance is dissolved or suspended in 200 ml of 0.1% w/v sterile peptone solution (pH 7.0–7.2) (Oxoid L34) and immediately filtered through one or more membrane filters (average pore diameter 0.45 μ or less).

Each filter is then washed three times, by passing 100 ml volumes of peptone solution through the membrane.

After filtration the membranes are transferred to tubes of media, containing at least 15 ml of Clausen Medium (Oxoid CM353) and tubes of Tryptone Soya Broth (soybean-casein digest medium) (Oxoid CM129). If only one filter is used, this is divided into two and the two halves placed in separate tubes.

Tubes of Clausen Medium (Oxoid CM353) are incubated for at least 14 days at 30–32° C.

Tubes of Tryptone Soya Broth (soybean-casein digest medium) (Oxoid CM129) are incubated for at least 14 days at 20–25° C.

METHOD II (DILUTION METHOD)

From each sample 1.0 ml of material or suspension is transferred to each of at least 10 tubes containing a minimum quantity of 15 ml of Clausen Medium (Oxoid CM353).

One half of the number of tubes is incubated at 30–32° C for at least 14 days and the other half at 20–25° C for the same time.

If the medium becomes turbid on incubation, subcultures must be taken as soon as possible. Subcultures must also be taken after normal incubation and observed for a further period of 14 days.

ASSESSMENT OF THE RESULTS

The standard microbial contamination test is passed if growth is not observed in any of the tubes. If growth is observed, the test may be repeated with twice the number of samples. The test is then passed if no growth is observed in any of these tubes. Growth is diagnosed by the appearance of turbidity in fluid or semi-fluid media, by the formation of colonies on solid media, or by microscopy of culture samples.

CONTROLS

Both methods of testing must be controlled for microbial inhibitors by adding a small inoculum of organisms (approximately 10 colony-forming bacteria) either to the tubes prepared in Method II or to peptone diluent, prior to filtration, in Method I.

If no growth occurs in the tubes containing the test organisms then the test must be repeated with the growth-inhibitory effect inactivated.

The test organisms recommended are:

Staphylococcus epidermidis (S. albus)
Clostridium sporogenes
Rhodotorula rubra

They are maintained on agar slants or deep agar stabs and the test inoculum is prepared from 24 hour cultures grown in Clausen Medium (Oxoid CM353) at 30–32° C. The *Rh. rubra* inoculum is prepared from a 48 hour culture grown in the same broth at 20–25° C.

Dithionite-Thioglycollate Broth was formulated by Clausen as a highly nutritious medium containing reducing agents and essential metals for the recovery of anaerobic spore-bearing organisms. It also contains lecithin and Tween 80 to overcome the effects of cationic agents which may show powerful bacteriostatic effects *in vitro.*

The broth should be prepared as directed and transferred to tubes or bottles in sufficient volumes (at least 15 ml) for the standard microbial-contamination test and rapidly cooled to 20° C after sterilization.

The medium is yellowish in colour and almost clear. It turns pale-pink under aerobic conditions. The upper third only of the medium should be pink by the time it is to be used. The medium should be stored in a cool place (not at 4° C) away from the light, for a maximum period of one month.

C.L.E.D. Medium

Code CM301

FORMULA

	grams per litre
Peptone (Oxoid L37)	**4**
'Lab-Lemco' Powder (Oxoid L29)	**3**
Tryptone (Oxoid L42)	**4**
Lactose	**10**
L-Cystine	**0.128**
Brom-thymol blue	**0.02**
Agar No. 1 (Oxoid L11)	**15**

pH 7.3 (approx.)

DIRECTIONS

Suspend 36.2 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring.

DESCRIPTION

A dehydrated Cystine-Lactose-Electrolyte Deficient (C.L.E.D.) medium made to the formula described by Mackey and Sandys (1966), as a modification for urinary bacteriology of the Electrolyte Deficient Medium developed by Sandys (1960).

This medium is recommended for urinary bacteriology, supporting the growth of all urinary pathogens and giving good colonial differentiations and clear diagnostic characteristics. The presence of important contaminants such as diphtheroids, lactobacilli and micrococci is also clearly elicited, giving an indication of the degree of contamination.

In the laboratory C.L.E.D. Medium provides a valuable non-inhibitory diagnostic agar for plate culture of urinary organisms. It is electrolyte deficient to prevent the swarming of Proteus species.

The medium has been used successfully in The Dip-inoculum Transport Medium technique (Mackey and Sandys 1965, 1966).

A variant of this technique has been described by Guttman and Naylor (1967), who employed media-coated slides.

These techniques overcome false bacteriological results associated with delay in the transport of the specimens of urine to the laboratory and permit a clinically accurate routine differential viable count. They are, therefore, suitable for both General Practitioner and hospital work including the screening of ante-natal specimens for symptomless bacteriuria.

For full details, the original papers should be consulted.

GROWTH CHARACTERISTICS on C.L.E.D. Agar (18 hours incubation)

E. coli—Yellow, opaque colonies with a slightly deeper coloured centre about 1.25 mm diam. (Non-lactose fermenting strains—blue colonies.)

Klebsiella sp.—Extremely mucoid colonies varying in colour from yellow to whitish/blue.

Proteus sp.—Translucent blue colonies usually smaller than E. coli.

Salmonella sp.—Flat blue colonies.

Ps. pyocyanea—Green colonies with typical matt surface and rough periphery.

Strep. faecalis—Yellow colonies about 0.5 mm diameter.

Staph aureus—Deep yellow colonies about 0.75 mm diameter, uniform in colour.

Coagulase negative Staphylococci—Pale yellow or white, more opaque than Strep. faecalis, often with paler periphery.

Corynebacteria—Very small grey colonies.

Lactobacilli—Similar to Corynebacteria but with a rougher surface.

REFERENCES

Mackey, J. P. and Sandys, G. H. (1965) 'Laboratory Diagnosis of Infections of the Urinary Tract in General Practice by means of a Dip-inoculum Transport Medium' *B.M.J.* **2**, 1286–1288.

Mackey, J. P. and Sandys, G. H. (1966) 'Diagnosis of Urinary Infections' *B.M.J.* **1**, 1173.

Sandys, G. H. (1960) *J. Med. Lab. Techn.* **17**, 224.

Guttman, D. and Naylor, G. R. E. (1967) 'Dip-Slide: an Aid to Quantitative Urine Culture in General Practice' *B.M.J.* **2**, 343–345.

C.L.E.D. Medium

(WITH ANDRADE INDICATOR)

Code CM 423

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**4**
'Lab-Lemco' Powder (Oxoid L29)		**3**
Tryptone (Oxoid L42)		**4**
Lactose		**10**
Cystine		**0.128**
Brom—thymol blue		**0.02**
Andrade Indicator		**0.1**
Agar No. 1 (Oxoid L11)		**15**

pH 7.5 (approx.)

DIRECTIONS

Suspend 36.2 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring.

DESCRIPTION

The formula for this medium is similar to that for C.L.E.D Medium, CM 301, but with the addition of acid fuchsin which enhances the colonial appearance and aids in identification of the organisms. The colour of the medium differs from that of the standard medium at various pH levels.

Bevis (1968) listed the pH and colour as follows:—

pH	**Colour of Medium**
7.4	Deep blue
7.0	Blue grey
6.8	Pale slate grey
6.6	Pinkish grey
6.4	Bright red with slight smokey tint
6.0	Bright red

The medium should not be incubated for longer than 24 hours since, if lactose permenters predominate, the whole of the medium may turn pink, masking the presence of non-lactose fermenters.

COLONIAL CHARACTERISTICS

E. coli—Bright pink semi-translucent colonies with a surrounding pink halo in the medium.

P. mirabilis—Blue-green translucent colonies.

K. aerogenes—Grey-green mucoid colonies.

A. anitratus—Small, grey-green, translucent colonies.

Staph. aureus—Smooth, entire, opaque; bright golden yellow colonies. Lactose fermenting.

Staph. albus—Smooth, entire, opaque porcelain white or very pale pink colonies.

Strep. faecalis—Similar to Staph. aureus but smaller and a much deeper orange yellow colour.

Strep. pyogenes—Small opaque grey-green colonies.

REFERENCE

Bevis, T. D. (1968) 'A modified electrolyte deficient culture medium'. *J. med. Lab. Technol.* **25**, 38–41.

Columbia Blood Agar Base

Code CM331

FORMULA

Special Peptone (Oxoid L72)	*grams per litre*	**23**
Starch		**1**
Sodium chloride		**5**
Agar No. 1 (Oxoid L11)		**10**

pH 7.3 (approx.)

DIRECTIONS

Suspend 39 g in 1 litre of distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C and add 5% sterile defibrinated blood.

DESCRIPTION

Traditionally blood agar bases have been either casein hydrolysate or meat infusion media. The advantage of the first lies in the rapid production of large colonies, and of the second in clearly defined zones of haemolysis and good colonial differentiation.

Columbia Agar Base (Ellner *et al.* 1966) combines the virtues of both these types of media to give an improved all-round performance.

This new base has shown versatility and superior performance in several applications.

Selective Medium for Gram positive cocci

With added colistin 10 µg/ml and nalidixic acid 15 µg/ml the blood agar provides an effective selective medium for gram positive cocci. *Proteus, Klebsiella* and *Pseudomonas* species are successfully inhibited while staphylococci, haemolytic streptococci and enterococci produce large clearly defined colonies.

'Chocolate' Agar for *Haemophilus influenzae*

Heated blood agar ('chocolate' agar) prepared with Columbia Agar Base produces larger colonies quicker than comparable preparations. Good growth of *Haemophilus influenzae* has been reported (Ellner *et al.* 1966).

Selective Medium for Neisseria (Thayer-Martin)

Thayer-Martin Medium can be prepared by the addition of colistin methane sulphonate (7.5 µg/ml) and vancomycin (3 units/ml) (Thayer and Martin 1966).

This medium is selective for *Neisseria gonorrhoeae* and *Neisseria meningitidis* and has shown itself to be an invaluable diagnostic agent. False positives are almost completely eliminated by the suppression of Mimeae and most other oxidase producing organisms.

The selective medium permits isolation of colonies for fermentation studies even from primary rectal cultures. Contaminated samples are easily purified on this medium for antibiotic sensitivity studies.

The efficiency of Thayer-Martin Medium makes possible the use of larger inocula in diagnostic procedures.

Virulence-test Medium for *C. diphtheriae*

Columbia Agar Base with added sterile serum provides an efficient *Corynebacterium diphtheriae* virulence test medium. After following the established technique, lines of toxin-antitoxin precipitation are clearly visible in 48 hours.

Base Medium without Enrichment

Used without added blood, Columbia Agar Base will support the growth of *Brucella abortus, Pasteurella pestis, Clostridium perfringens* and all commonly occurring Enterobacteriaceae.

REFERENCES

Ellner, P. D., Stoessel, C. J., Drakeford, E. and Vasi, F. (1966) 'A new culture medium for medical bacteriology' *Tech. Bull. Reg. Med. Techn.* **36**, No. 3, *reprinted in Amer. J. Clin. Path,* (1966) **45,** 502–504.

Thayer, J. D. and Martin, J. E. (1966) 'An improved medium for the cultivation of *N. gonorrhoeae and N. meningitidis' Publ. Hlth. Report* **81**, 559-562.

Complement Fixation Test Diluent Tablets

Code BR16

FORMULA

Barbitone	*grams per litre*	**0.575**
Sodium chloride		**8.5**
Magnesium chloride		**0.168**
Calcium chloride		**0.028**
Barbitone Soluble		**0.185**

pH 7.2

DIRECTIONS

Dissolve 1 tablet in 100 ml of warm distilled water.

DESCRIPTION

Complement fixation tests are extensively employed in the diagnosis of many different diseases, including infections due to viruses as well as bacteria.

These tablets are intended for the simple and convenient preparation of the special diluent for use in complement fixation tests. This diluent, a modification of that described by Whillans (1950), is similar to the diluent employed by Mayer *et al.* (1950). It is superior to the physiological saline formerly used because it contains calcium and magnesium, and is of the correct pH for optimum results.

Mayer *et al.* (1946) investigated the effect of cations on the haemolytic function of complement, by the addition of a number of substances to the veronal buffered saline base. They were able to conclude:

(i) Some divalent cations, especially Mg^{++}, are essential for the haemolytic action of complement.

(ii) The haemolytic system does not contain sufficient Mg^{++} for optimal haemolytic activity, so that a marked enhancement can be obtained by the addition of extra Mg^{++}. The enhancing effect of tissue fluids can be ascribed to their contribution of Mg^{++}.

(iii) The anticomplementary effect of some substances can be overcome by the addition of extra Mg^{++}.

(iv) Ca^{++} may also be essential to the haemolytic process but its action is much less pronounced than that of Mg^{++}.

Bradstreet and Taylor (1962) recommended the use of Oxoid Complement Fixation Test Diluent Tablets in a description of complement fixation tests for virus diseases (Fulton and Dumbell, 1949).

REFERENCES

Bradstreet, C. M. Patricia and Taylor, C. E. D. (1962) 'Technique of Complement-Fixation Test Applicable to the Diagnosis of Virus Diseases' *Mon. Bull. Min. Hlth. Pub. Hlth. Lab. Serv* **21**, 96–104.

Fulton, F. and Dumbell, K. R. (1949) 'The Serology of Influenza Viruses *J. gen. Microbiol.* **3,** 97.

Mayer, M. M. *et al.* (1946) 'Activating Effect of Magnesium and other Cations on the Hemolytic Function of Complements' *J. Exp. Med.* **84**, 535–548.

Whillans, D. (1950) *J. clin. Path.* **3**, 57.

Cooked Meat Medium

Code—Granules CM81
Tablets CM82

Peptone (Oxoid L37)	*grams per litre*	**10**
'Lab-Lemco' Powder (Oxoid L29)		**10**
Neutral heart tissue		**30**
Sodium chloride		**5**

pH 7.4 (approx.)

DIRECTIONS

Granules: Add 55 g to 1 litre of distilled water and soak for 15 minutes. Distribute into narrow containers keeping the meat in suspension so that a uniform layer is obtained. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 10 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

Tubes of correctly prepared and distributed medium each contain an equal thickness of meat particles overlaid by clear supernatant liquid. If a greater depth of meat particles is required, employ 1.1 grams of granules or 2 tablets to each 10 ml of water.

Tablets are particularly convenient for the preparation of small quantities of medium and ensure the even distribution of meat particles. For the preparation of tubes of medium containing more than 10 ml use a proportionately greater number of tablets or a proportionately heavier weight of granules.

DESCRIPTION

Cooked Meat Medium (a modification of Robertson medium) is a well established substrate for the cultivation and maintenance of anaerobic and aerobic organisms. It is particularly suitable for the cultivation and differentiation of *Clostridium* species, and stock cultures remain viable for several years.

Oxoid Cooked Meat Medium may be used for the isolation of *Clostridium perfringens* (Williams Smith, 1959a); during the investigation of *Clostridium perfringens* phages (Williams Smith, 1959b); as an enrichment medium for staphylococci (Williams Smith and Crabb, 1960a and b); for the characterization of cellulolytic soil anaerobes (Skinner, 1960).

TECHNIQUE

Cooked Meat Medium may be used for cultivation of aerobes, anaerobes and organisms with intermediate oxygen requirements. It is particularly valuable because of its capacity to initiate growth from minute inocula.

Anaerobic Culture. It is preferable to use the freshly reconstituted and sterilized medium, which is inoculated as soon as it has cooled to approximately 37° C. Tubes of Cooked Meat Medium which are not used immediately on cooling should be placed in a boiling water bath in order to remove dissolved oxygen, cooled (without agitation), and then inoculated.

Clostridia may be divided into two main groups by their action upon the medium (Cruickshank, 1960):

Saccharolytic organisms—rapid production of acid and gas, but no digestion of the meat. The cultures may have a slightly sour smell, and the meat is often reddened.

Proteolytic organisms—proteolytic ferments decompose and blacken the meat with the formation of foul-smelling sulphur compounds. The protein is broken up into amino-acids, and the small, white, feathery masses of tyrosine crystals may also be seen.

For the isolation of anaerobes, it is important to use solid media in conjunction with Cooked Meat Medium.

Aerobic Culture. No seal is required, the tube of medium is incubated with the cap loose. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium.

Maintenance. Cooked Meat Medium is excellent for the maintenance of stock cultures of aerobic and anaerobic organisms. Cultures (particularly those of spore-forming species) remain viable when stored at room temperature for many months. Stock organisms should be subcultured once or twice a year to establish purity.

REFERENCES

Cruickshank, R. (1960) 'Mackie and McCartney's Handbook of Bacteriology' **10th** ed., Livingstone Ltd., London.
Skinner, F. A. (1960) 'The Isolation of Anaerobic Cellulose-decomposing Bacteria from Soil' *J. gen. Microbiol.* **22**(2), 539–554.
Williams Smith, H. (1959a) 'The Effect of the Continuous Administration of Diets Containing Tetracycline and Penicillin on the Number of Drug-Resistant and Drug-Sensitive *Clostridium welchii* in the Faeces of Pigs and Chickens' *J. Path. Bact.* **77**(1), 79–93.
Williams Smith, H. (1959b) 'The Bacteriophages of *Clostridium perfringens*' *J. gen. Microbiol.* **21**(3), 622–630.
Williams Smith, H. and Crabb, W. E. (1960a) 'Antibiotic Sensitivity of Staphylococci from Noses of Veterinary Surgeons and Farmers' *Lancet* **2,** 515–516.
Williams Smith, H. and Crabb, W. E. (1960b) 'The Effect of Diets Containing Tetracyclines and Penicillins on the *Staphylococcus aureus* Flora of the nose and Skin of Pigs and Chickens and their Human Attendants' *J. Path. Bact.* **79**(2), 243–249

Cooked Meat Medium (SYNTHETIC)

Code—Granules CM 439
Tablets CM 440

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10**
'Lab Lemco' Powder (Oxoid L29)		**10**
Textured Soya Protein		**30**
Sodium chloride		**5**

pH 7.0 (approx)

DIRECTIONS

Granules: Using a calibrated scoop, distribute 0.6 g amounts of granules into tubes or bottles and add 10 ml of distilled water. Increase the weight of granules and volume of water proportionately for larger quantities. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water in a narrow container. Soak for 5 minutes and sterilize by autoclaving at 121° C for 15 minutes.

Tubes of correctly prepared medium contain an equal depth of particles overlaid by clear supernatant fluid.

DESCRIPTION

Cooked Meat Medium (Synthetic) is particularly useful because of its capacity to initiate growth from minute inocula.

It is based on the medium of Robertson (1916) and makes use of the advances in textured vegetable protein to provide a reliable medium of good appearance and performance. This medium is recommended for the cultivation and maintenance of anaerobic and aerobic organisms as well as for those with intermediate oxygen requirements.

Cooked Meat Medium (Synthetic) grows a wider range of organisms than Cooked Meat Medium, (CM81), with better sulphide blackening of the particles and a greater density of growth.

TECHNIQUE

Anaerobic culture. It is preferable to use freshly reconstituted and sterile medium which is inoculated as soon as it has cooled to approximately 37° C. Tubes which are not used on the day of preparation should be placed in a boiling water bath or steamer for about 15 minutes to remove dissolved oxygen. They should be allowed to cool without agitation and then inoculated.

Clostridia may be divided into two main groups by their action on the medium.

Saccarolytic organisms

There is rapid production of acid and gas but no digestion of the soya protein. Cultures may have a slightly sour smell.

Proteolytic organisms

Proteolysis causes decomposition of the soya protein with the formation of foul-smelling sulphur compounds and blackening of the protein. However, some saccharolytic strains also produce H_2S and will blacken the protein, but to a lesser degree.

Aerobic culture. The tube of medium is incubated with the cap loose and no seal is required. Aerobes grow at the top whilst more anaerobic species grow deeper in the medium.

REFERENCES

Robertson, M. (1916) 'Notes upon certain anaerobes isolated from wounds' *J. Path. Bact.* **20,** 327–349.

Corn Meal Agar

Code—Powder CM103
Tablets CM104

FORMULA

Corn Meal Extract (from 50 grams whole maize)	*grams per litre*	2
Agar No. 3 (Oxoid L13)		15
pH 6.0 (approx.)		

DIRECTIONS

Powder: Suspend 17 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Corn Meal Agar is a well established mycological medium which is a suitable substrate for chlamydospore production by *Candida albicans* (*Monilia albicans*) and the maintenance of fungal stock cultures.

When grown on this medium, microscopic examination of *Candida albicans* shows the characteristic chlamydopsore production which is an accepted criterion for the identification of this species. Prospero and Reyes (1955) investigated the use of corn meal agar, soil extract agar, and purified polysaccharide medium for the morphological identification of *C. albicans.* Out of 290 yeast colonies isolated on Sabouraud agar, corn meal agar stimulated the production of chlamydospores in 149 colonies (51%), soil extract agar in 103 (36%) and purified polysaccharide medium in 94 (32%).

The addition of Tween 80 (e.g. 1%) to Corn Meal Agar greatly enhances the development of chlamydospores on the medium (e.g. Rosenthal and Furnari, 1958; Kelly and Funigiello, 1959; Walker and Huppert, 1959; Walker *et al.*, 1960; Gordon and Little, 1962–63).

Mackenzie (1962) found that all 163 isolates of *Candida albicans* obtained from laboratories in the United Kingdom produced chlamydospores on Oxoid Corn Meal Agar but Dawson (1962), using only 27 isolates of *Candida albicans,* found that Oxoid Czapek Dox Agar and rice infusion agar were slightly superior for chlamydospore production (see page 102).

Corn meal agar is a nutritionally impoverished medium and so may be employed for the maintenance of stock cultures of fungi, especially the black-pigmented varieties.

TECHNIQUE

A single Petri dish containing Corn Meal Agar may be used to identify four or five different colonies of *Candida* grown on Sabouraud Dextrose Agar (CM41). Using a straight wire, pick a colony off the surface of the latter medium and make a deep cut in the Corn Meal Agar (i.e. a horizontal furrow). Repeat for each colony. Place a flamed sterile coverslip over the line of inoculum. After incubation for 24 to 48 hours at 22° C, the steaks are examined microscopically, through the cover slip, using a low power objective. Along such streaks, *C. albicans* produces mycelium-bearing, ball-like clusters of budding cells and the characteristic thick-walled, round chlamydospores (Conant *et al.*, 1955).

REFERENCES

Conant, N. F., Smith, D. T., Baker, R. D., Callaway, J. L. and Martin, D. S. (1955) 'Manual of Clinical Mycology' 2nd ed., W. B. Saunders Company, Philadelphia and London. p. 185.
Dawson, Christine O. (1962) 'Identification of *Candida albicans* in Primary Culture *Sabouraudia* 1(4), 214–219.
Gordon, M. A. and Little, G. N. (1962–63) 'Effective Dehydrated Media with Surfactants for Identification of *Candida albicans*' *Sabouraudia* **2,** 171–175.
Kelly, J. P. and Funigiello, F. (1959) '*Candida albicans* A Study of Media Designed to Promote Chlamydospore Production' *J. Lab. clin. Med.* **53,** 807–809.
Mackenzie, D. W. R. (1962) 'Serum Tube Identification of *Candida albicans*' *J. clin. Path.* **15(6),** 563–565.

Prospero, Magdalene T and Reyes, A. C. (1955) 'The Efficacy of Corn Meal Agar, Soil Extract Agar and Purified Polysaccharide Medium in the Morphological Identification of *Candida albicans*' *Acta Med. Phillipina* **12**(2), 69–74.
Rosenthal, S. A. and Furnari, D. (1958) 'Slide Agglutination as a Presumptive Test in the Laboratory Diagnosis of *Candida albicans*' *J. Invest. Derm.* **31**, 251–253.
Walker, L. and Huppert, M. (1959) 'A Rapid Reliable Technique for the Identification of *Candida albicans*' *Am. J. clin. Path.* **31**, 551–558.
Walker, L., Huppert, M. and Woods, A. (1960) Corn Meal-Tween Agar: An improved medium for the identification of *Candida albicans*' *Am. J. clin. Path.* **33**, 190–194.

Crossley Milk Medium

Code CM213

FORMULA

Skim Milk Powder (Oxoid L31)	*grams per litre*	**100.0**
Peptone (Oxoid L37)		**10.0**
Bromo-cresol purple		**0.1**

pH 6.8 (approx.)

DIRECTIONS

Cream 110 g of the powder with a little distilled water and gradually dilute to 1 litre with continuous mixing. Tube in 10 ml quantities and autoclave at 121° C for 5 minutes.

DESCRIPTION

A simple medium originally described by Crossley (1941) for the routine examination of canned food samples for anaerobic bacteria.

This medium was evolved as the result of comparative trials carried out by Crossley with several standard media. It is capable of giving rapid growth without the use of special anaerobic apparatus, yet the bacteria detected may be provisionally identified by their reactions upon the medium.

Crossley milk medium is recommended, in the second edition of Tanner's 'The Microbiology of Foods' (1944), for the examination of meat, meat products, and canned foods for sporing anaerobes.

TECHNIQUE

The following method of examination is suggested: Inoculate 10 ml of Oxoid Crossley Milk Medium with 1.5 to 2.0 g of the sample. Incubate for 3 to 4 days at 37° C and examine for the following striking and characteristic reactions:

Reaction	Organism Indicated
1. Neutral or alkaline pH (purple colour), gas production, soft curd followed by rapid digestion of casein, often to a clear brown liquid, formation of black sediment accompanied by typical foul odour.	*Cl. putrificum* *Cl. sporogenes* *Cl. flabelliferum* *Cl. oedematiens* *Cl. histolyticum*
2. No initial change of pH, formation of soft curd within 2–3 days, slight gas formation. Complete digestion later with alkaline reaction, no odour.	*Cl. centrosporogenes*
3. Slight acidity (pale yellow colour), formation of soft curd, and whey. Slight gas production.	*Cl. sphenoides*
4. Acid (bright yellow colour), formation of firm clot and gas. Bleaching of the indicator may sometimes occur.	*Cl. butyricum*
5. (a) Acid, formation of 'stormy' clot. (b) Acid and 'stormy' clot but with less gas and cloudy whey.	(a) *Cl. welchii* (*Cl. perfringens*) (b) Usually *Cl. tertium*
6. Strong alkaline pH, with peptonization commencing at the surface and spreading downwards. Digestion not complete, no blackening, no odour, no gas production.	*B. subtilis*, *B. vulgatus*
7. Acid and clot, or slightly acid only. Peptonization in some cases.	*B. cereus*, *B. coagulans*, *B. silvaticus* and various cocci (More detailed tests required)

Crossley (1941) modified his medium, by the addition of 20% (w/v) of autoclaved meat or fish paste, so that it was suitable for the examination of vegetable and dairy products.

The medium recommended by Jepsen (1957) in 'Meat Hygiene' published by the World Health Organization, is Crossley Milk medium modified by the addition of 20% (w/v) of cooked fish; it is suitable for the examination of meat products for clostridia, and gives diagnostic reactions essentially similar to those outlined above. Riemann (1959) modified Crossley milk medium by the addition of 0.08% of cysteine hydrochloride, before autoclaving, or by the addition of 1 ml of a sterile 10% sodium thioglycollate solution just before use.

Any of the above additions may be used to supplement Oxoid Crossley Milk Medium.

REFERENCES

Crossley, E. L. (1941) 'The Routine Detection of Certain Spore-Forming Anaerobic Bacteria in Canned Foods' *J. Soc. Chem. Ind.* **60**, 131–136.
Riemann, H. (1959) Personal communication.
Tanner, F. W. (1944) 'The Microbiology of Foods' 2nd ed., Garrard Press, London, pp. 893, 1001–1002.
Jepsen, A. (1957) Albertsen, V. E. *et al.* 'Meat Hygiene' World Health Organization, Geneva, pp. 425–426, 439.

Czapek Dox Agar (MODIFIED)

Code—Powder CM97
Tablets CM98

FORMULA

Sodium nitrate	*grams per litre*	2.0
Potassium chloride		0.5
Magnesium glycerophosphate		0.5
Ferrous sulphate		0.01
Potassium sulphate		0.35
Sucrose		30.0
Agar No. 3 (Oxoid L13)		12.0
pH 6.8 (approx)		

DIRECTIONS

Powder: Suspend 45.4 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115° C for 20 minutes. Mix well before pouring. If it is required to adjust the reaction to pH 3.5 add 10 ml of Lactic Acid 10% (SR21) per litre after sterilization.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 115° C for 20 minutes. To adjust to pH 3.5 for acidophilic organisms, aseptically add 0.05 ml of Lactic Acid 10% SR21 to each 5 ml of medium after sterilization.

DESCRIPTION

Czapek Dox Agar (Modified) is a semi-synthetic medium containing sodium nitrate as the sole source of nitrogen. It is one of the most useful solid media for the general cultivation of fungi. It has the advantage of being of defined chemical composition. In the Oxoid medium magnesium glycerophosphate and potassium sulphate replace the magnesium sulphate and potassium phosphate of the original. This modification prevents the precipitation of magnesium phosphate. The medium is also a highly satisfactory substrate for chlamydospore production by *Candida albicans* (Dawson, 1962).

Dawson (1962) employed Oxoid Czapek Dox Agar (Modified) in a new technique for the identification of *Candida albicans* by chlamydospore formation in primary culture, using swabs taken from the mouth and from the vagina. Identification was usually possible within 24 hours. The Oxoid medium showed good chlamydospore production whereas the original formulation did not. After 24 hours' incubation 23 out of 27 *C. albicans* strains had formed chlamydospores on Oxoid Czapek Dox Agar (Modified), 21 on rice infusion agar, 10 on Oxoid Corn Meal Agar and 10 on a corn meal agar made in the laboratory. After 48 hours 25 strains had formed chlamydospores on both the Oxoid medium and the rice agar, 24 on Oxoid Corn Meal Agar and 20 on the laboratory medium. Dawson concluded that the Oxoid Czapek Dox medium and the rice infusion agar were the most satisfactory media. None of 14 strains of unidentified yeasts formed chlamydospores on any medium.

Smith (1960) cited the following recommendations for the use of Czapek Dox Agar for taxonomic studies: by Thom and Church (1926) for *Aspergillus;* by Thom (1930) and by Raper and Thom (1949) for *Penicillium;* and by Waksman (1931) for Actinomycetes.

TECHNIQUE

General Cultivation

To avoid excessive condensation cool the molten medium to 50° C before pouring approximately 12 ml into each 9 cm diameter Petri dish. Store the poured plates in an inverted position and inoculate using needle or wire, with the plate still inverted in order to avoid scattering stray fungal spores over the surface of the medium. Time and temperature of incubation vary considerably according to the species being cultivated, as a general guide, incubate for 1–2 weeks at 25° C. Most *Penicillium* species have an optimum growth temperature between 20° and 25° C, whilst many *Aspergillus* species grow best at about 30° C. However, different fungi grow over a wide range of temperatures—*Aspergillus fumigatus* grows well at 50° C (Smith, 1960) and *Cladosporium herbarum* will grow on meat at − 6° C (Brooks and Kidd, 1921; Brooks and Hansford, 1922).

Identification of *Candida albicans* (Dawson, 1962)

1. Using an inoculating needle (previously flamed, cooled and rubbed against the swab) cut across and through the medium in a Czapek Dox Agar plate to the base of the Petri dish. With the same needle, raise the medium along the whole of one side of the cut—so that the inoculum is spread between the agar and the base of the dish.

2. Incubate the inoculated plates for 24 hours at 28° C.

3. Using a low-power objective, microscopically examine the unopened plates for chlamydospores through the base of each dish. Alternatively, remove the tops of the dishes, and examine through the top of the medium.

4. If no chlamydospores are seen, incubate for a further 24 hours and re-examine.

REFERENCES

Brooks, F. T. and Hansford, C. G. (1922) Mould Growths upon Cold-store Meat' *Trans. Brit. Mycol. Soc.* **8,** 113–142.

Brooks, F. T. and Kidd, M. N. (1921) 'Black Spot of Chilled and Frozen Meat' *Specia. Report No. 6,* Food Invest. Board, DSIR, London.

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Czapek Dox Liquid Medium
(MODIFIED)

Code CM95

FORMULA

	grams per litre
Sodium nitrate	2.0
Potassium chloride	0.5
Magnesium glycerophosphate	0.5
Ferrous sulphate	0.01
Potassium sulphate	0.35
Sucrose	30.0

pH 6.8 (approx.)

DIRECTIONS

Add 33.4 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 115° C for 20 minutes.

DESCRIPTION

A semi-synthetic fluid medium for the cultivation of fungi and bacteria capable of utilizing sodium nitrate as the sole source of nitrogen.

D.C.L.S. Agar

Code CM393

FORMULA

	grams per litre
Special Peptone (Oxoid L72)	10.0
Sodium citrate	10.5
Sodium thiosulphate	5.0
Lactose	5.0
Sucrose	5.0
Sodium desoxycholate	2.5
Neutral Red	0.03
Agar No. 3 (Oxoid L13)	12.0

pH 7.2 (approx.)

DIRECTIONS

Suspend 50 g in 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Cool to 50° C and pour plates. DO NOT AUTOCLAVE.

DESCRIPTION

D.C.L.S. Agar is a modified form of Desoxycholate Citrate Agar which includes sucrose in its formulation. The addition of this fermentable carbohydrate increases the usefulness of the medium because non-pathogenic sucrose-fermenting organisms may be recognised by their red colonies, e.g. some Proteus species and para-colon organisms.

D.C.L.S. Agar reduces the number of false-positive sub-cultures when picking colonies and therefore improves the efficiency of isolation.

The Special Peptone, used in D.C.L.S. Agar, includes the nucleic acid factors, vitamins and carbon compounds of meat extract, as well as a rich variety of polypeptides. It has improved the growth of shigellae and salmonellae, but it should be noted that *Sh. sonnei* may exhibit a translucent, pink colony which however, cannot be confused with the red *Esch. coli* colony.

The selectivity of D.C.L.S. Agar is similar to Desoxycholate Citrate Agar and it will grow *Vibrio* species, as well as salmonellae and shigellae, whilst inhibiting the growth of *Esch. coli*.

D.C.L.S. Agar may be inoculated directly from the specimen, or inoculated after enrichment through Selenite Broth, Muller-Kauffmann Tetrathionate Broth (CM343) or Tetrathionate Broth (CM29). The plates should be incubated overnight (18–24 hours) at 35° C and examined for the presence of pale, translucent or colourless colonies. Sub-cultures can be made into confirmatory media such as Kohn Two-Tube Medium (CM179) or Triple Sugar Iron Agar (CM277) or picked for transfer to nutrient broth for subsequent motility tests and serological agglutinations.

Defibrinated Blood

See page 65.

Desoxycholate Agar

Code—Powder CM163
Tablets CM164

FORMULA

	grams per litre
Peptone (Oxoid L37)	10.0
Lactose	10.0
Sodium desoxycholate	1.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.0
Ferric citrate	1.0
Sodium citrate	1.0
Neutral red	0.03
Agar No. 3 (Oxoid L13)	15.0

pH 7.3 (approx.)

DIRECTIONS

Powder: Suspend 45 g in 1 litre of distilled water. Bring to the boil over gauze and flame to dissolve the medium completely. Agitate to prevent charring.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Raise to boiling point in a water bath to dissolve completely.

THIS MEDIUM IS HEAT SENSITIVE: AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE OR REMELT.

DESCRIPTION

Desoxycholate Agar is a differential medium for the direct count of coliforms in dairy products (American Public Health Association, 1967). It may also be employed for the isolation of enteric pathogens from rectal swabs, faeces, or other specimens.

The medium may be used in a 'pour-plate' technique or as a surface inoculated medium. A thin layer of uninoculated desoxycholate agar poured over the surface of a gelled 'pour-plate' assists subsequent counting.

TECHNIQUE

Enumeration of Coliforms in Milk and Cream (APHA, 1967)

1. Pipette 1–4 ml of the sample (or decimal dilution of the sample) into a sterile Petri dish.

2. Cool freshly prepared Desoxycholate Agar to 42–44° C and add 10–20 ml to each dish.

3. Mix the contents of the dishes by gentle tilting and rotation.

4. Allow the plates to solidify and pour on an overlay of 3–4 ml of uninoculated Desoxycholate Agar.

5. When the overlay has set, invert the plates and incubate them for 18–24 hours at 35° C.

6. Count all dark red colonies measuring at least 0.5 mm in diameter, and calculate the number of coliform colonies per millilitre or gram of original sample.

Isolation of Enterobacteriaceae

It is advisable to use Desoxycholate Agar in parallel with other plating media for this purpose.

Lightly inoculate a Desoxycholate Agar plate with faeces, rectal swab, or enrichment culture. Incubate for 18–24 hours at 37° C and examine. Non-lactose fermenters of enteric origin form colourless colonies. Non-lactose fermenters which are not of enteric origin are generally inhibited by the sodium desoxycholate in the medium. Identify suspect colonies in the usual manner.

REFERENCE

American Public Health Association (1967) 'Standard Methods for the Examination of Dairy Products' 12th ed., APHA Inc., New York, pp. 58-59.

Desoxycholate Citrate Agar

Code—Powder CM35
Tablets CM36

FORMULA

'Lab-Lemco' Powder (Oxoid L29) . *grams per litre*	5.0
Peptone (Oxoid L37)	5.0
Lactose	10.0
Sodium citrate	5.0
Sodium thiosulphate	5.0
Ferric citrate	1.0
Sodium desoxycholate	2.5
Neutral red	0.025
Agar No. 3 (Oxoid L13)	15.0

pH 7.0 (approx.)

DIRECTIONS

Powder: Suspend 48.5 g in 1 litre of distilled water. With frequent agitation bring to the boil over a gauze and flame to dissolve completely. Mix well and pour plates immediately. Dry the agar surface before use.

Tablets: Add 1 tablet to 15 ml of distilled water and soak for 5 minutes. Bring to the boil in a water bath to dissolve completely. Pour plates immediately.

THIS MEDIUM IS HEAT SENSITIVE: AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE OR REMELT.

DESCRIPTION

An Oxoid modification of Leifson medium, for the isolation and maximum recovery of intestinal pathogens. It is less selective and inhibiting than Desoxycholate Citrate Agar (Hynes) but colonial characteristics are identical on the two media—see page 82. CM35 provides an opaque background against which one may more easily discern the clearing produced by alkali-producing pathogens. On the other hand, Desoxycholate Citrate Agar (Hynes) is a clear medium and assists the examination of colonial morphology by transmitted light.

Desoxycholate Citrate Agar

(HYNES)

Code CM227

FORMULA

'Lab-Lemco' Powder (Oxoid L29) . *grams per litre*	5.0
Peptone (Oxoid L37)	5.0
Lactose	10.0
Sodium citrate	8.5
Sodium thiosulphate	5.4
Ferric citrate	1.0
Sodium desoxycholate	5.0
Neutral red	0.02
Agar No. 3 (Oxoid L13)	12.0

pH 7.3 (approx.)

DIRECTIONS

Suspend 52 g in 1 litre of distilled water. Bring to the boil over gauze and flame, to dissolve completely. Agitate to prevent charring. Dry the agar surface before use.

THIS MEDIUM IS HEAT SENSITIVE: AVOID EXCESSIVE OR PROLONGED HEATING DURING RECONSTITUTION. DO NOT AUTOCLAVE OR REMELT.

DESCRIPTION

An improved medium, based on the Hynes (1942) modification of Leifson medium for the isolation of salmonellae and shigellae. As a consequence of improvements in manufacture it has been possible to eliminate, in the majority of cases, the occurrence of 'sticky' *Shigella sonnei* colonies which hitherto have often been difficult to emulsify for slide agglutination tests. Colonies of *Shigella sonnei* are larger and more numerous. They may easily be picked off and can be readily emulsified in saline for slide agglutination tests.

Desoxycholate Citrate Agar (Hynes) is more selective than CM35, in particular, CM227 is more inhibitory to coliform and *Proteus* species.

TECHNIQUE

Inoculate the medium heavily with faeces or rectal swabs, spreading part of the original inoculum in order to obtain well separated colonies on some portion of the plate. Incubate for 18–24 hours at 37° C. If organisms are late developers or if no non-lactose fermenters are observed, incubate for a further 24 hours.

Colonies may be picked directly off the medium for serological and biochemical tests. It should be noted that *Escherichia coli* survives on the medium even though it does not usually grow—therefore colonial purity should be established by subculture on to a differential but less inhibitory medium, e.g. MacConkey Agar CM7.

COLONIAL CHARACTERISTICS

(Following incubation at 37° C)

The medium is clear and pale pink. Lactose fermenting organisms produce pink colonies and may be surrounded by a zone of precipitated desoxycholic acid, which is due to acid production. The colonies of non-lactose fermenters are colourless, and due to their alkaline reaction they are surrounded by a clear orange-yellow zone of medium.

Escherichia coli—most strains are inhibited, but the few strains which grow produce pink umbilicated colonies 1–2 mm in diameter which may be surrounded by a zone of precipitation. *Aerogenes* colonies are domed and mucoid.

Shigella sonnei—the colonies grow from 1 mm diameter after 18 hours' incubation to 2 mm after 38 hours; they are smooth and initially colourless, becoming pale pink on further incubation due to late lactose fermentation.

Shigella flexneri—colonies are colourless and similar in appearance to those of *Shig. sonnei*, but often with a narrow plane periphery round a central dome.

Salmonella paratyphi B—from 1 mm diameter after 18 hours' incubation to 2–4 mm on the second day, when they are slightly opaque, dome-shaped, with a central black dot.

Salmonella typhosa—¼ to 1 mm in diameter after 18 hours and pale pink, a day later they are flat, conical, 2 mm in diameter, colourless and slightly opaque, often with a central grey dot.

Other *Salmonella* colonies—similar to those of *Salm. paratyphi B*.

Non-pathogenic non-lactose fermenters, such as *Proteus* and *Pseudomonas* species, grow on the medium and may produce colonies which closely simulate those of the salmonellae or shigellae. *Proteus* colonies are often glossy (more translucent than those of the pathogens), with a large central black dot and a 'fishy' odour.

REFERENCE

Hynes, M. (1942) 'The Isolation of Intestinal Pathogens by Selective Media' *J. Path. Bact.* **54**, 193–207.

Dextrose

Code L71

A special bacteriological grade of dextrose (glucose) for use in culture media.

Dextrose 10%

Code SR11

Filter sterilized solution, available in 5 ml ampoules, in boxes of ten.

Dextrose Broth

Code CM175

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**3**
Tryptose (Oxoid L47)		**10**
Dextrose		**5**
Sodium chloride		**5**

pH 7.2 (approx.)

DIRECTIONS

Add 23 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.

For the cultivation of organisms which require a low Eh, if the reconstituted medium has been stored prior to use, remove dissolved oxygen by placing the tubes in a boiling water bath for 15 minutes and cool without agitation before inoculation.

DESCRIPTION

A highly nutritious broth especially suitable for the initial isolation of micro-organisms which are fastidious, attenuated, or only present in small numbers. The 0.5% of dextrose acts as a nutrient and also as a reducing agent—thus rendering the medium particularly suitable for the cultivation of those organisms which require a reduced Eh. Even so, the medium may be improved for the growth of both anaerobes and aerobes by the addition of a small proportion of agar (0.1–0.2%) most conveniently added during reconstitution as Oxoid Agar Tablets CM49 (1 tablet per 100 ml).

Dextrose Broth is recommended for use as a blood culture medium and for sterility testing. It is particularly suitable for the initiation of growth but it is important to subculture after growth takes place since many bacteria will not survive for long in the acid conditions produced by dextrose fermentation (Stokes, 1955).

REFERENCES

Stokes, E. Joan (1955) Clinical Bacteriology' Edward Arnold Ltd., London, p. 27.

Dextrose Peptone Agar

Code CM13

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**20**
Dextrose		**10**
Sodium chloride		**5**
Agar No. 3 (Oxoid L13)		**15**

pH 7.2 (approx.)

DIRECTIONS

Suspend 50 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Dextrose Peptone Agar is recommended for the general cultivation of organisms, and makes an excellent basal agar medium for the preparation of glucose blood agar, etc. In deep tubes or in special Petri dishes it will support good growth of anaerobes. It is particularly suitable for the detection and cultivation of thermophilic organisms associated with 'flat-sour' spoilage in canned goods, and for this purpose a suitable indicator should be added to the melted medium before sterilization.

Dextrose Peptone Broth

Code CM11

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**20**
Dextrose		**10**
Sodium chloride		**5**

pH 7.2 (approx.)

DIRECTIONS

Add 35 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A simple broth, with a carbohydrate source, for use in routine sterility testing. For this purpose Dextrose Peptone Broth CM11 has largely been superseded by the thioglycollate media described in British and American pharmacopoeias—see page 232.

For fermentation studies with dextrose and with other sugars, see page 217.

Dextrose Tryptone Agar

Code—Powder CM75
Tablets CM76

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**10.0**
Dextrose		**5.0**
Bromo-cresol purple		**0.04**
Agar No. 3 (Oxoid L13)		**12.0**

pH 6.9 (approx.)

DIRECTIONS

Powder: Suspend 27 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A bacteriologically controlled medium for the detection and enumeration of thermophilic and mesophilic organisms in food products, etc.

Dextrose tryptone agar, evolved as the result of several years research by Williams (1936), is most suitable for the cultivation and enumeration of the thermophilic bacteria causing 'flat-sour' spoilage of canned food. Its use for routine cultural purposes is recommended by Cameron (1936) and the Association of Official Agricultural Chemists (1945). Dextrose tryptone agar is also recommended by:

(i) Tanner (1944) for the examination of canned food, sugar, and starch for thermophilic bacteria of the *Bacillus stearothermophilus* type (i.e. 'flat-sour' spoilage bacteria).

(ii) The American Public Health Association (1953) for the enumeration of mesophilic and thermophilic aerobic bacteria in sweetening agents used in frozen dairy foods.

(iii) The National Canners Association (1956) for determination of the total plate and 'flat-sour' count of thermophilic bacteria spores in ingredients, such as sugar and starch.

(iv) The American Public Health Association (1958) for the enumeration of mesophilic organisms and 'flat-sour' spores in sugars, starches and other complex carbohydrates; and for the enumeration of 'flat-sour' thermophiles in cereals and cereal products, dehydrated fruits and vegetables, and spices.

(v) Baumgartner and Hersom (1956) for the examination of low and medium-acid canned food (above pH 4.5) for 'flat-sour' thermophiles, mesophilic aerobes, and facultative anaerobes.

Bashford (1948) reported that the addition of ½–1% of meat extract greatly improves the medium.

Townsend *et al.* (National Canners Association, 1954) showed that some batches of bromo-cresol purple are more inhibitory than others, but this variability is overcome in the Oxoid medium by large-scale production and stringent biological control.

TECHNIQUE

The instructions given below are included only as an indication of the mode of use of Dextrose Tryptone Agar, and will vary according to the original sample and the exact purpose of the investigation. For more exact details of technique it is advisable to consult one of the standard manuals mentioned in the references.

Enumeration of Mesophiles:

Into each of 5 Petri dishes, pipette dilutions of the sample to be tested. Cover and mix the inoculum with sterile Dextrose Tryptone Agar and incubate for 72 hours at 32° C. Count the total number of colonies, with separate totals for acid producing (yellow halo) and non-acid producing colonies.

Enumeration of 'Flat-Sour' Thermophiles:

Inoculate as above and incubate for 48 hours at 55° C. 'Flat-sour' colonies (e.g. *Bacillus stearothermophilus*) are typically round, 2–5 mm in diameter, with an opaque centre, and surrounded by a yellow zone in contrast with the purple medium.

REFERENCES

American Public Health Association (1953) 'Standard Methods for the Examination of Dairy Products' 10th ed. APHA Inc., New York, p. 189 and 200.
American Public Health Association (1958) 'Recommended Methods for the Microbiological Examination of Foods' APHA Inc., New York, p. 48–49 and 52, 58, 69, 130 and 172.
Association of Official Agricultural Chemists (1945) 'Official and Tentative Methods of Analysis', Washington.
Bashford, T. E. (1948) Personal Communication.
Baumgartner, J. G. and Hersom, A. C. (1956) 'Canned Foods' 4th ed., Churchill Ltd., London, pp. 229–230 and 247.
Cameron, E. J. (1936) 'Report on Culture Media for (the Bacteriological Examination of) Nonacid (Food) Products' *J. Assoc. Official Agr. Chem.* **19**, 433–438
National Canners Association (1954) 'A Laboratory Manual for the Canning Industry' 1st ed., National Canners Association, Washington.
National Canners Association (1956) Ibid., 2nd ed., pp. 2–9.
Nuffield Provincial Hospitals Trust (1958) 'Present Sterilizing Practice in Six Hospitals' NPHT, London, pp. 58–59.
Tanner, F. W. (1944) 'The Microbiology of Foods' 2nd ed., Garrard Press, Champaers, pp. 762–763 and 1127–1128.
Williams, O. B. (1936) 'Tryptone Medium for the Detection of Flat-Sour Spores' *Food Res.* **1**(3), 217–221.

Dextrose Tryptone Broth

Code—Powder CM73
Tablets CM74

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**10.0**
Dextrose		**5.0**
Bromo-cresol purple		**0.04**

pH 6.9 (approx.)

DIRECTIONS

Powder: Add 15 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Dextrose Tryptone Broth is widely recommended for the aerobic cultivation and detection of many different organisms causing spoilage in canned foods and other products.

The American Public Health Association (1958) and Baumgartner and Hersom (1956) recommended this formulation for the bacteriological examination of low- and medium-acid canned foods (pH 4.5 and above); both methods include inoculation of 10 ml amounts of the broth with one or two grams of the food product. For food products in this pH range, the suggested procedure is aerobic cultivation in Dextrose Tryptone Broth in parallel with anaerobic cultivation in other media, Oxoid Liver Broth prepared from tablets (Code CM78) is most suitable for this purpose. Duplicate sets of tubes are incubated at 37° C and at 55° C. Organisms which produce acid from dextrose, such as *Bacillus stearothermophilus* and other 'flat-sour' organisms, are detected by the colour change of the medium from purple to yellow.

REFERENCES

American Public Health Association (1958) 'Recommended Methods for the Microbiological Examination of Foods' APHA Inc., New York, pp. 38 and 173.
Baumgartner, J. G. and Hersom, A. C. (1956) 'Canned Foods 4th ed., Churchill Ltd. London, pp. 229–230 and 247.

Diagnostic Sensitivity Test Agar

DST Agar
Code CM261

FORMULA

Proteose Peptone (Oxoid L46)	*grams per litre*	**10.0**
Veal infusion solids		**10.0**
Dextrose		**2.0**
Sodium chloride		**3.0**
Disodium phosphate		**2.0**
Sodium acetate		**1.0**
Adenine sulphate		**0.01**
Guanine hydrochloride		**0.01**
Uracil		**0.01**
Xanthine		**0.01**
Aneurine		**0.00002**
Agar No. 1 (Oxoid L11)		**12.0**

pH 7.4 (approx.)

DIRECTIONS

Suspend 40 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

For blood agar, cool the base to 50° C and add 7% of Defibrinated Horse Blood SR50. Mix with gentle rotation and pour into Petri dishes (12 ml for a 9 cm dish) or other containers. RECONSTITUTION AND MIXING SHOULD BE PERFORMED IN A FLASK AT LEAST 2½ TIMES THE VOLUME OF MEDIUM TO ENSURE ADEQUATE AERATION OF THE BLOOD.

DESCRIPTION

A sensitivity test agar, containing a minimal amount of dextrose, which may be used as a blood agar for the determination of haemolytic reactions. Oxoid Diagnostic Sensitivity Test Agar fulfils the World Health Organization (1961) criteria for a sensitivity test medium. When enriched with blood it will support the growth of delicate pathogens such as *Neisseria meningitidis, Streptococcus pneumoniae*, alpha- and beta-haemolytic streptococci, and *Haemophilus influenzae*. For less demanding organisms such as micrococci, shigellae, salmonellae, coliform bacteria, and *Proteus* species—the medium may be employed without blood. None of the ingredients of the medium will interfere with the action of antibiotics and chemotherapeutic agents such as sulphonamides—the latter produce well defined zones of inhibition due to the absence of neutralizing substances.

The gelling agent, 'Ionagar' No. 2, produces a clear firm medium which permits superior diffusion of large molecule antibiotics (Bechtle and Scherr, 1958).

Media containing dextrose at a concentration of 1% w/v are known to be unsuitable for the determination of haemolytic reactions. However, Marshall and Kelsey (1960) have shown that small concentrations of dextrose (such as the 0.2% w/v in the Oxoid medium) improve bacterial growth without interfering with haemolytic reactions.

TECHNIQUE

Performance of the sensitivity test:

1. Evenly inoculate the surface of the medium with the specimen to be tested or with a broth subculture of an organism. A sterile swab or bent glass rod will assist even distribution. The density of the inoculum should be gauged to yield adjacent but not confluent colonies. If the clinical specimen is heavily infected it is recommended that a suitable dilution in sterile saline be made before inoculating the plate.

2. Dry the surface of the plate until there is no visible moisture.

3. Place a Multodisk, or a number of single discs equally spaced, on to the inoculated surface.

4. Incubate for 18 hours at 37° C, and examine for the presence or absence of zones of inhibition radiating from the impregnated tips of the Multodisk, or around the single discs.

See page 169 for details of the Multodisk.

REFERENCES

Bechtle, R. M. and Scherr, G. H. (1958) 'A New Agar for in vitro Antimicrobial Sensitivity Testing' *Antibiotics and Chemotherapy* 8(12), 599–606.

Expert Committee on Antibiotics (1961) World Health Organization Technical Report Series No. 210 'Standardization of Methods for Conducting Microbic Sensitivity Tests WHO, Geneva.

Marshall, J. H. and Kelsey, J. C. (1960) 'A Standard Culture Medium for General Bacteriology' *J. Hyg.*, Camb. 58, 367–372.

Dip Slide

Code DS2

The Oxoid Dip Slide represents one of the most significant advances in the diagnosis of urinary tract infections introduced in recent years.

The principle is simple. An agar coated slide is provided in a sterile screw cap container. The slide is attached to the cap. By dipping the slide into freshly voided urine reliable counts can be obtained, showing the numbers of organisms present in the urine *at the time of voiding*.

For full details of technique and interpretation of results, please ask for the separate Oxoid leaflet.

DNase Agar

Code CM321

FORMULA

Tryptose (Oxoid L47)	*grams per litre*	**20**
Deoxyribonucleic acid		**2**
Sodium chloride		**5**
Agar No. 3 (Oxoid L13)		**12**

pH 7.3 (approx.)

DIRECTIONS

Suspend 39 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

The ability of coagulase-positive staphylococci to split deoxyribonucleic acid (DNA) provides the basis for a convenient, diagnostic laboratory test for the identification of potentially pathogenic organisms.

The DNA molecule is hydrolysed to a mixture of mono- and poly-nucleotides by the action of enzymes (DNases) produced by the organisms.

Deoxyribonucleic acid contained in Oxoid DNase Agar is attacked by microbial DNase. After incubation the plates are flooded with Normal hydrochloric acid which reacts with nucleic acid in the medium and forms a cloudy precipitate. DNase producing colonies are surrounded by a clear area containing nucleotide fractions which are not precipitated by acid.

Increased DNase activity of staphylococci isolated from clinical material has been described by Weckman and Catlin (1957) and Jeffries, Holtman and Guse (1957). Both these publications demonstrated a close correlation of DNase activity with coagulase production.

DiSalvo (1958) observed perfect correlation between DNase and coagulase activity using *Staphylococcus aureus* from clinical specimens. The results are shown in Table 1.

Table 1

Organism Source	*No. Tested*	*DNase* +	*DNase* −	*Coagulase* +	*Coagulase* −
Blood	2	2	0	2	0
Urine	6	5	1	5	1
Respiratory Tract	119	119	0	119	0
Exudates	76	73	3	73	3
Faeces	1	0	1	0	1
Total	204	199	5	199	5

Blair, Emerson and Tull (1967) tested 2,415 suspect *Staphylococcus aureus* colonies. They found that all the 1,385 coagulase positive strains were also DNase positive. Further tests showed that 223 colonies which demonstrated positive DNase activity were coagulase-negative. (See Table 2.)

Table 2

DNase and coagulase reactions	*No./Total Tested*
DNase+ Coagulase+	1385/2415 (57.3%)
DNase+ Coagulase−	223/2415 (9.3%)
DNase− Coagulase−	807/2415 (33.4%)

From these results they concluded that the test could be implemented to screen out coagulase-negative organisms.

TECHNIQUE

Inoculate the plates by spotting the organism onto the surface of the agar so that a thick plaque of growth is evident after 18 hours incubation. Flood the plates with Normal HCl and look for clearing around the colonies (DNase positive).

REFERENCES

Blair, E. B., Emerson, J. S. and Tull, A. H. (1967) 'A New Medium, Salt Mannitol Plasma Agar, for the Isolation of *Staphylococcus aureus*' *Am. J. Clin. Path.* **47**, 30-39.
DiSalvo, J. W. (1958) 'Deoxyribonuclease and Coagulase Activity of Micrococci' *Med. Techns. Bull Suppl. to U.S. Armed Forces Medical Journal* **9**, 191-196.

Dorset Egg Medium

Code PM5

FORMULA

Fresh egg mixture (yolks and whites)		**750 ml**
'Lab-Lemco' Powder (Oxoid L29)	*grams*	**2.5**
Peptone (Oxoid L37)		**2.5**
Sodium chloride		**1.25**
Crystal violet		**q.s.**

and distilled water to 1 litre

DESCRIPTION

An inspissated egg medium for the cultivation of *Mycobacterium* species. This modification, described by Mackie and McCartney (1956), does not contain glycerol, and is therefore suitable for the cultivation of *M. bovis* as well as *M. tuberculosis.* Not only does the crystal violet content of the medium serve to suppress contaminants, but it also renders *M. bovis* colonies more easily visible.

This medium is also available without crystal violet as PM5p.

REFERENCE

Mackie, T. J. and McCartney, J. E. (1956) 'Handbook of Practical Bacteriology' 9th ed., Livingstone Ltd., London, pp. 177–178.

Dulbecco Solution

See page 221

Dulcitol 5%

Code SR15

Filter sterilized solution, available in 5 ml ampoules, in boxes of ten.

Earle Solution

Code BR21

FORMULA

Sodium chloride	*grams per litre*	**6.8**
Potassium chloride		**0.4**
Calcium chloride		**0.2**
Magnesium sulphate		**0.2**
Sodium dihydrogen phosphate		**0.125**
Dextrose		**1.0**

DIRECTIONS

Dissolve the contents of this sachet in 1 litre of de-ionised (or double distilled) water and sterilize by autoclaving for 20 minutes at 115° C. Cool to room temperature. To adjust the reaction to pH 7.2–7.4, aseptically add 44 ml of a sterile solution of sodium bicarbonate 5% w/v. Phenol red indicator may be included, if required, with the bicarbonate solution.

DESCRIPTION

A balanced salt solution formulated by Earle (1943) which is used as a basis for tissue culture media, and as a rinse and diluent in tissue culture procedures. Earle solution differs from other balanced salt solutions such as Hanks, in being designed to equilibriate with 5% carbon dioxide. It is a widely used buffered salt solution which serves to maintain pH and osmotic pressure whilst also providing an adequate concentration of essential inorganic ions (Paul, 1959).

Earle, Gey, and Hanks solutions are all modifications of Tyrode solution in which the phosphates are closer to physiological levels. Earle solution contains the most bicarbonate and therefore has the highest buffering capacity (Parker, 1961). The total molarity of molecules and ions in both Earle solutions and human plasma is 0.307 M (Stewart and Kirk, 1954).

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Edwards Medium (MODIFIED)

Code CM27

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**10.0**
Peptone (Oxoid L37)		**10.0**
Aesculin		**1.0**
Sodium chloride		**5.0**
Crystal violet		**0.0013**
Thallous sulphate		**0.33**
Agar No. 3 (Oxoid L13)		**15.0**

pH **7.4** (approx.)

DIRECTIONS

Suspend 41 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115° C for 20 minutes. Cool to 50° C, add 5 to 7% of sterile bovine or sheep blood, mix well and pour plates.

DESCRIPTION

A selective medium for the rapid isolation of *Streptococcus agalactiae* and other streptococci involved in bovine mastitis.

Crystal violet or gentian violet and thallium salts have long been used in selective media for streptococci. Haxthausen (1927) employed a selective crystal violet medium for the isolation of skin streptococci. Bryan (1932), using gentian violet blood agar, found that the growth of saprophytic milk bacteria was prevented whilst that of streptococci was unaffected. Edwards (1933) employed a crystal violet aesculin blood agar for the cultural diagnosis of bovine mastitis, whilst McKenzie (1941) used a medium containing thallium acetate for the same purpose.

The Oxoid medium depends for its selective action on the presence of crystal violet and thallous sulphate. The few resistant organisms usually produce black colonies, due to aesculin fermentation, whilst mastitis streptococci grow well and form lightly coloured colonies.

TECHNIQUE

Inoculate the surface of the medium with centrifuged deposits from milk samples and incubate at 37° C. Mastitis streptococci may be α, β- of μ haemolytic. Pure cultures may be obtained from single colonies and identified by further tests.

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EE Broth

Code CM317

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10.0**
Dextrose		**5.0**
Disodium hydrogen phosphate anhyd.		**6.45**
Potassium dihydrogen phosphate		**2.0**
Ox Bile purified		**20.0**
Brilliant Green		**0.0135**

pH **7.2** (approx.)

DIRECTIONS

Dissolve 43.5 grams in 1 litre of distilled water. Distribute 100 ml quantities in 250 ml flasks and heat at 100° C for 30 minutes only. Cool rapidly in cold running water. This medium is heat sensitive and MUST NOT BE AUTOCLAVED.

DESCRIPTION

EE Broth (Buffered glucose—Brilliant Green-bile broth) is recommended as an enrichment medium for *Enterobacteriaceae* in the bacteriological examination of foods (Mossel, Visser and Cornelissen, 1963) and animal feedstuffs (Van Schothurst, Mossel, Kampelmacher and Drion, 1966). This medium is more inhibitory to non-*Enterobacteriaceae* than other non selective media e.g. Mannitol broth (Taylor 1961) or Lactose broth (North 1961), by virtue of the presence of brilliant green and bile salts in the preparation.

Mossel and Harrewijn (1972) stated that the enumeration of *Enterobacteriaceae* is of great importance in monitoring the sanitary quality of food and drugs but that the reliability of the methods used depends upon resuscitation of damaged cells. Such weakened cells may arise from exposure to dehydration, low pH and other unfavourable conditions.

They established that incubation for 2 hours in well-aerated Tryptone Soya Broth (Oxoid CM 129) at 25° C should precede enrichment in EE broth. This procedure is recommended for dried foods (Mossel and Ratto, 1970), animal feeds (Mossel, Shennan and Vega, 1973) and semi-preserved foods (Mossel and Ratto, 1973). Occasionally, with a particular dry product, a longer incubation period—but never over eight hours of resuscitation—is necessary.

Oxoid EE Broth was formulated to overcome the unsatisfactory effects of inhibition on small numbers of *Enterobacteriaceae* cells due to bile salt variations. The inclusion of purified ox bile eliminated these problems and a preliminary assay can be used to check growth by inoculating approximately one viable cell per medium unit.

For the bacteriological evaluation of processed foods the entire enterobacteriaceae group can be used as indicator organisms (Mossel 1973). This will overcome the discrepancies that can arise when lactose-negative, anaerogenic lactose-positive or late lactose fermenting Enterobacteria are present but are missed by the standard 'coli-aerogenes' tests. To overcome these problems lactose media have been replaced by those containing glucose. Mossel et al. (1963) cited several examples in the literature which referred to various foods contaminated with salmonellae, although results for coliforms were negative. A more recent example quoted by Mossel (1973) involved an outbreak of diarrhoea caused by French mould-fermented soft cheese contaminated by *Escherichia coli* serotype 0124. This organism is lactose-negative and therefore was not detected in coliform tests but only recognised when the commodity was tested for *Enterobacteriaceae* since it fermented glucose rapidly.

EE Broth should be used as an enrichment broth in conjunction with MacConkey glucose agar (Oxoid Violet Red Bile Dextrose Agar CM323). When specific organisms, rather than *Enterobacteriaceae* in general are required, subcultures must be made onto lactose differential media e.g. Oxoid Desoxycholate Citrate Agar (CM 35), Oxoid Brilliant Green Agar (CM 329), or MacConkey Agar (CM7) for the detection of lactose-negative or delayed organisms.

Sample size should not be less than 10 grams to yield the organisms being sought.

TECHNIQUE

1. Resuscitate debilitated cells by incubating 1:10 dilutions of the food samples under investigation in Oxoid Tryptone Soya Broth (CM 129) at 25° C for 2–8 hours. The fluid layer should not be much deeper than one centimetre. Shake the flask to disperse the contents alternately in clockwise and anti-clockwise directions for 30 seconds on three successive occasions.

2. After the period of time necessary for resuscitation, ten-fold volumes of EE broth are added to the resuscitated suspensions.
3. Shake to disperse as above. For large samples it is desirable to add the resuscitation medium containing the product under examination, to equal volumes of double strength EE Broth.
4. Incubate at 30° C for 18 to 24 hours.
5. The enrichment cultures are stabbed into freshly steamed deep tubes of Violet Red Bile Dextrose Agar (CM 323), cooled to 30° C. After 18 to 24 hours incubation at 30° C the tubes are inspected for the typical growth pattern of *Enterobacteriaceae,* i.e. development of an inoculation line reaching to the bottom of the tube, surrounded by a coaxial cylinder of purple precipitate with or without gas production. (see page 281).

Positive cultures may be examined for oxidase activity to preclude their being *Aeromonas* species. Where closer identification of isolated *Enterobacteriaceae* is required growth in Kligler Iron Agar, I.M.V.E.C. tests (E = Eijkman test), Urea deamination and Lysine decarboxylation tests may be applied (Ref. Oxoid Manual).

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Egg Yolk Emulsion

Code SR47

DESCRIPTION

A sterile stabilised emulsion of egg yolk for use in culture media. Egg yolk media have been described for a number of different purposes (see the references for details) but in all cases the preparation of the sterile egg yolk emulsion is both difficult and time-consuming. For this reason, Oxoid Egg Yolk Emulsion was developed. It may be added directly to nutrient media for the identification of *Clostridium, Bacillus* and *Staphylococcus* species by their lecithinase activity. Oxoid Egg Yolk Emulsion is particularly suitable for inclusion in Baird Parker egg yolk tellurite medium as the Emulsion is stable and provides a suitably opaque agar against which zones of clearing can be easily discerned. See Egg Yolk Tellrite Emulsuion (Oxoid SR54).

TECHNIQUE

Examination of Bacteria for Lecithinase

For demonstration of lecithinase activity (especially in the investigation of 'bitty cream' conditions) add 0.5 or 1.0 ml of the Emulsion to 10 ml of sterile prepared Blood Agar Base CM55 or Nutrient Broth No. 2 CM67—in both cases to clear the medium, raise the final salt concentration by the addition of 1% of sodium chloride. After incubation for up to 5 days at 37° C, lecithinase-producers render the broth opalescent, whilst, on the solid medium, their colonies are surrounded by zones of opacity. Organisms producing potent phospholipinase (e.g. *Bacillus cereus*) render the broth thickly milky after 8–10 hours, and a thick curd usually appears after 16–24 hours. Some *Bacillus* species produce a positive reaction more slowly (e.g. *B. anthracis*) whilst others produce no opalescence in the medium.

Gillespie and Alder (1952) found that, for the examination of staphylococci, opacity production was increased by the addition of 1% of dextrose to their egg yolk medium.

Detection of 'Bitty-Cream' Organisms in Milk and Water

'Bitty-Cream' spoilage of milk is caused by the lecithinase produced by *Bacillus cereus* and *Bacillus cereus* var. *mycoides*. This type of curdling is more prevalent in hot weather, and pasteurization may only serve to increase the spoilage. The causative organisms may be readily detected as follows:

(i) *Raw and Pasteurized Milk*

A modification of the method described by Stone and Rowlands (1952). Mix the specimen well and transfer 10 ml portions to sterile $\frac{5}{8}$ in. (16 mm) test-tubes. Heat in a water bath at 80° C for 10 minutes, cool to 21° C and incubate for 40 to 42 hours at 21° C.

After incubation, streak a small loopful of the cream from each tube on to an Egg Yolk Agar plate,* incubate at 30° C and examine for colonies giving a zone of opacity. Pick off these colonies into tubes of 10 ml of Egg Yolk Broth and incubate for 24 hours at 30° C. A heavy white curd in the broth denotes a positive reaction.

After the primary incubation, leave the Egg Yolk Agar plates for a further 24 hours at room temperature—more colonies may develop zones of opacity, and are picked off into Egg Yolk Broth for confirmation.

For pasteurized milk, it is also advisable to streak some of the cream on to Egg Yolk Agar before heating at 80° C, since positive results are sometimes obtained by direct streaking but not after heating. Certain strains of *Bacillus cereus* give a positive reaction in Egg Yolk Broth after 6 hours' incubation. These 'fast' reactors may tend to produce 'bitty cream' in pasteurized milk under commercial conditions rather than the 'slow' reactors which need 24 hours' incubation to give a positive reaction.

(ii) *Waters, Churn Rinses, Washed Bottle Rinses*

Plate 5 ml and 1 ml samples on Oxoid Milk Agar CM21. Incubate for 24 to 48 hours at 30° C and pick off colonies resembling aerobic spore-bearers into Egg Yolk Broth for confirmation as with milk samples.

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* Add 1 ml Egg Yolk Emulsion SR47 to 10 ml sterile Blood Agar Base CM55 containing an additional 10 g salt per litre.

Also, aseptically pipette 10 ml volumes of the samples into $\frac{5}{8}$ in. (16 mm) test-tubes. Heat in a water bath at 80° C for 10 minutes and then prepare Milk Agar pour-plates inoculated with 1 ml and 5 ml of the sample. Incubate for 24 to 48 hours at 30° C and treat any suspicious colonies as above. In this case there is no need to incubate the heated samples before plating out.

Use of Egg Yolk Broth as a Neutralizing Sampling Fluid

Morris and Darlow (1959) employed egg yolk broth containing 0.1% (v/v) of Tween 20, as a neutralizing sampling fluid for the testing of bactericidal paints. The bactericides were dissolved in the basal paint, usually in 10% (w/w) concentration, and brushed on to cardboard. The coated cardboard was dried, cut into squares, which were stored, and exposed to atomized suspensions of the test bacteria. Each exposed square was then immersed in 10 ml of sampling fluid and shaken to remove deposited organisms. A small quantity of bactericide was washed out of the paints by this method, but it was found that the Tween-egg yolk broth neutralized twice the amount of the most active bactericides used that could be washed out in the time allowed. Immediately after shaking, portions of the diluent were plated out by the Miles and Misra (1938) method, and a colony count performed after 18 hours' incubation at 37° C.

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Bacillus

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Egg Yolk Tellurite Emulsion

Code SR54

DIRECTIONS

Add 50 ml to 1 litre of Baird-Parker Medium (CM275).

(50 ml Egg Yolk Tellurite Emulsion contains the equivalent of 3 ml of 3.5% potassium tellurite. This is the amount recommended for 1 litre of Baird-Parker Medium. i.e. concentration in SR54 is 0.21% w/v. Final conc. in Baird-Parker Medium is 0.01% w/v.)

DESCRIPTION

A sterile emulsion of egg yolk containing potassium tellurite for use in Baird-Parker Medium (Oxoid CM275).

This stabilised emulsion extends the life of a Baird-Parker Medium plate from a few days to one month.

Baird-Parker Medium is widely used in the food industry for the detection of pathogenic staphylococci, but hitherto it has suffered from the limitation that the plates once prepared had to be used within one to three days.

The Oxoid preparation overcomes this limitation which was, in fact, due to instability in the egg yolk emulsion. The physical process of stabilisation has been shown not to affect the diagnostic egg-yolk changes characteristic of the Baird-Parker Medium.

Baird-Parker plates incorporating Oxoid's Egg Yolk Tellurite Emulsion can be stored for up to one month at 4° C, but they must be protected from moisture loss by enclosure in plastic or other vapour proof packaging.

REFERENCE

Holbrook, R., Anderson, Judy M. and Baird-Parker, A. C. (1969) 'The Performance of a Stable Version of Baird-Parker's Medium for Isolating *Staph. aureus*' *J. appl. Bact.* **32**, 187–192

Electrophoresis Strips

Oxoid Electrophoresis Strips are composed of pure microporous cellulose acetate membrane. This material has many advantages over filter paper as a supporting medium for electrophoresis and may also be used as a substrate in diffusion techniques. The use of this material was originally investigated and introduced by Dr. J. Kohn of Queen Mary's Hospital, Roehampton, and was demonstrated by him at the Bruges 1957 colloquium on the Proteins of Biological Fluids.

Use of Oxoid Strips in United Kingdom and overseas laboratories has established them as a superior medium for a variety of electrophoretic techniques, and their application to immuno-diffusion for demonstration of antibody-antigen reactions has shown them to be equal, if not superior, to an agar gel.

Some of the advantages of Oxoid Electrophoresis Strips are listed below:

(i) The material is relatively pure.

(ii) Adsorption is minimal and there is practically no tailing.

(iii) Sharp separation: in particular the albumin from alpha fractions which may be difficult to separate on paper.

(iv) Fractions not seen on paper electrophoresis may be demonstrated.

(v) The strips can be rendered completely transparent to both visible and ultra-violet light, making them eminently suitable for scanning.

(vi) The bands may be completely dissolved in suitable organic solvents for quantitative determinations.

For full details of technique and an extensive bibliography, please ask for the special Oxoid monograph 'Oxoid Electrophoresis Strips'.

Endo Agar

CM37

This medium has been withdrawn as the powdered dye-medium may constitute a health hazard. A leaflet explaining Oxoid's reasons for this decision is available on request.

See **ENDO AGAR BASE CM479**

Endo Agar Base

Code CM479

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10.0**
Lactose		**10.0**
Potassium phosphate		**3.5**
Sodium sulphite		**2.5**
Agar No. 1 (Oxoid L11)		**10.0**

pH 7.5 (approx.)

DIRECTIONS

Suspend 36 g in 1 litre of distilled water. Add 4 ml of a 10% W/V alcoholic solution of basic fuchsin. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring.

BASIC FUCHSIN IS A POTENTIAL CARCINOGEN AND CARE SHOULD BE TAKEN TO AVOID INHALATION OF THE POWDERED DYE AND CONTAMINATION OF THE SKIN.

Plates should be stored in the dark to preserve their pale pink colour.

DESCRIPTION

Endo Agar is a long established medium which was originally devised for the isolation of the typhoid bacillus. More reliable media for this purpose have since been evolved, and the medium is now used for the differentiation of lactose fermenting and non-lactose fermenting intestinal organisms, particularly during confirmation of the presumptive test for coliforms. Production of both acid and aldehyde by lactose fermenting organisms, such as *Escherichia coli*, gives rise to the characteristic red colouration of the colony and the surrounding medium.

Endo agar is recommended by:

(i) The American Public Health Association (1953) for use in the bacteriological examination of dairy products. Tubes of liquid media showing a positive presumptive reaction for coliforms are subcultured on to Endo agar. The formula of Oxoid Endo Agar corresponds to Endo Agar Formula II in the APHA publication.

(ii) The APHA, American Water Works Association, and the Federation of Sewage and Industrial Wastes Associations (1955) for confirmation of the presumptive test for coliform organisms, during the bacteriological examination of water.

(iii) The APHA (1958) for the confirmation of the presumptive test for coliforms, during the bacteriological examination of food.

(iv) Windle Taylor (1958) for the isolation and differentiation of *coli-aerogenes* bacteria by the plating method, following positive presumptive tests with liquid media.

TECHNIQUE

For the confirmation of presumptive tests with liquid media, subculture tubes showing gas, or acid and gas formation, onto an Endo Agar plate. Incubate for 24 hours at 35° C or 37° C (American and British procedures respectively).

Lactose fermenting coliforms (e.g. *Escherichia coli*) give rise to deep red colonies which colour the surrounding medium and possess a golden metallic sheen.

Non-lactose fermenters form colourless translucent colonies, against the pink to colourless medium.

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Eosin Methylene Blue Agar (LEVINE)

Code—Powder CM69
Tablets CM70

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10.0**
Lactose		**10.0**
Dipotassium hydrogen phosphate		**2.0**
Eosin Y		**0.4**
Methylene blue		**0.065**
Agar No. 3 (Oxoid L13)		**15.0**

pH 6.8 (approx.)

Powder: Suspend 37.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 60° C and shake the medium in order to oxidize the methylene blue (i.e. restore its blue colour) and to suspend the precipitate which is an essential part of the medium.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 60° C and swirl the medium in order to oxidize the methylene blue (i.e. restore its blue colour), and to suspend the precipitate which is an essential part of the medium.

DESCRIPTION

This versatile medium, originally developed by Levine (1918 and 1921), is used for the differentiation of *Escherichia coli* and *Aerobacter aerogenes*, for the rapid identification of *Candida albicans*, and for the identification of coagulase-positive staphylococci.

The medium is recommended by the American Public Health Association (1950, 1953, 1955, 1958), the Society of American Bacteriologists (1957) and by Windle Taylor (1958) for the detection, enumeration or differentiation of members of the coliform group.

Weld (1952, 1953) proposed the use of Levine eosin methylene blue agar, with added chlortetracycline hydrochloride, for the rapid identification of *Candida albicans* in clinical materials. A positive identification of *Candida albicans* could be made after 24 to 48 hours incubation at 37° C in 10% carbon dioxide from faeces, oral and vaginal secretions, and nail or skin scrapings. Vogel and Moses (1957) confirmed the reliability of Weld's method for the relatively rapid identification of *C. albicans* in sputum. They found that use of eosin methylene blue agar was just as reliable as more conventional methods for the identification of this organism in sputum. In addition, the medium provided a means for the identification of several Gram-negative genera. Doupagne (1960) also investigated the use of the Levine medium under tropical conditions.

Haley and Stonerod (1955) found that Weld's method was variable so that Walker and Huppert (1959) advocated the use of corn meal agar and a rapid fermentation test in addition to the Levine medium. Using the combined rapid technique they were able to obtain results within 48 to 72 hours.

Subsequent to the findings of Vogel and Moses (1957), Menolasino *et al.* (1960) used Levine eosin methylene blue agar for the identification of coagulase-positive staphylococci. Both *albus* and *aureus* strains of coagulase-positive staphylococci grew as characteristic colourless, pin-point colonies. The Levine medium was more efficient than tellurite glycine agar and showed good correlation with the plasma coagulase test.

COLONIAL CHARACTERISTICS

Escherichia coli—isolated colonies, 2–3 mm diameter, with little tendency to confluent growth, exhibiting a greenish metallic sheen by reflected light and dark purple centres by transmitted light.

Aerobacter aerogenes—4–6 mm diameter, raised and mucoid colonies, tending to become confluent, metallic sheen usually absent, grey-brown centres by transmitted light.

Non-lactose fermenting intestinal pathogens—translucent and colourless.

Candida albicans—after 24 to 48 hours at 37° C in 10% carbon dioxide 'spidery' or 'feathery' colonies. Other *Candida* species produce smooth yeast-like colonies. Since a typical appearance is variable it is advisable to use a combined method such as that of Walker and Huppert (1959).

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Fildes Extract

Fildes Peptic Digest of Blood

Code SR46

DESCRIPTION

Oxoid Fildes Extract is prepared by the action of the enzyme pepsin on defibrinated sheep blood at optimum temperature and pH value, as described by Fildes (1920). It is a rich source of growth factors, including haematin and coenzyme, derived from the blood cells from which it is prepared. As some of the growth factors are thermolabile it should not be heated above 55° C.

The Extract is supplied preserved with chloroform in screw-capped bottles. Before use, hold at 55° C for 30 minutes to remove the chloroform, but take care not to exceed this temperature. Add to the appropriate medium only after the latter has been sterilized.

TECHNIQUE

Fildes Extract is recommended for the preparation of many culture media among which are the following examples:

Nagler Medium

For the identification of *Clostridium perfringens* (*Cl. welchii*) and *Cl. bifermentans* (Hayward, 1941; Stokes, 1955a):

Melt 100 ml sterile nutrient agar (Blood Agar Base CM55 is recommended) and cool to 50° C. Add 5 ml of Fildes Extract and 20 ml sterile human serum or plasma. Pour plates and dry. If human serum is unavailable, Concentrated Egg Yolk Emulsion SR47 may be used in 5% concentration. Mark the Nagler plate into halves with a grease pencil. On one half spread a few drops of *Cl. welchi* antitoxin of British Pharmacopoeia strength (polyvalent therapeutic antitoxin may also be used) and allow to dry. Spread the swab over both halves of the plate, spreading the inoculum in the same pattern on each side. Incubate in an anaerobic jar. The reaction is positive if cloudy zones are seen around colonies on the half of the medium without antitoxin, and if similar colonies on the antitoxin half show no zones.

Nagler medium is also stated to grow organisms of the *Mycoplasma* (PPLO) species and the addition of 5 to 10% CO_2 appears to aid primary isolation (Stokes, 1955b; Edward, 1954).

Fildes Broth (Fildes Peptic Blood Broth)

Add 5 ml of Fildes Extract to 100 ml of sterile Nutrient Broth No. 2 (CM67) or other liquid nutrient media.

Fildes Agar (Fildes Peptic Blood Agar)
Melt 100 ml of nutrient agar or Blood Agar Base (CM55), cool to 50° C and add 5 ml of Fildes Extract. Prepare sloped tubes—it is an advantage to keep them until the surface of the medium is dry at the top of the slope. For the isolation of *Cl. tetani* (Fildes, 1925), incubate the specimen anaerobically in peptic blood broth for two to four days at 37° C. Heat the culture for 30 minutes at 65° C to kill non-sporing spreaders, e.g. *Proteus.*

The condensation water of a peptic blood agar slope is inoculated from the heated culture, and the tube is incubated anaerobically. After 24 to 48 hours the edge of the culture is examined with low power magnification, when a growth of tetanus bacilli is seen as a mass of very fine filaments. Subcultures from the marginal growth usually yield pure cultures of *Cl. tetani* (Mackie and McCartney, 1953).

Fildes broth and agar, which are transparent and have the colour of nutrient broth or agar, give copious growths of *Haemophilus influenzae* and are admirably suited for the primary isolation of this organism (Wilson and Miles, 1964).

Willis (1960) recommended the use of Fildes extract in the following media:

Milk Agar (Reed and Orr, 1941)—for the detection of strong proteolytic activity by anaerobes.

Human Serum Agar (Hayward, 1941, 1943)—see above under Nagler medium.

Egg Yolk Agar (McClung and Toabe, 1947)—also for the identification of clostridia, the reactions on this medium are more consistent and stronger than on Human Serum Agar. Oxoid Egg Yolk Emulsion SR47 can also be used for the preparation of this medium, see page 95.

Lactose-Egg-Yolk Milk Agar (Willis and Hobbs, 1959)—a medium for the identification of anaerobes which, in addition to serving as a half antitoxin-Nagler plate, also demonstrates lactose fermentation and proteolysis. Egg Yolk Emulsion (see page 95) is recommended for use in the preparation of the medium.

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G.C. Agar Base

Code CM367

FORMULA

	grams per litre
Special Peptone (Oxoid L72)	**15.0**
Corn Starch	**1.0**
Sodium chloride	**5.0**
Dipotassium hydrogen phosphate	**4.0**
Potassium dihydrogen phosphate	**1.0**
Agar No. 1 (Oxoid L11)	**10.0**

pH 7.2 (approx.)

Sterile G.C. Supplement

Code SR56

FORMULA (*per vial*)

Yeast Fractions	**5.0 g**
Dextrose	**0.75 g**
Sodium bicarbonate	**0.075 g**
Vancomycin	**1,500 µg**
Colistin methane sulphonate	**3,750 µg**
Nystatin	**6,250 units**
Trimethoprim	**2,500 µg**

Sterile G.C. Supplement

(without antibiotics)

Code SR62

FORMULA (*per vial*)

Yeast Fractions	**5.0 g**
Dextrose	**0.75 g**
Sodium bicarbonate	**0.075 g**

Soluble Hæmoglobin Powder

Code L53

A specially prepared powder which will form a solution at 2% w/v in water and a stable suspension after sterilization.

Isolation Techniques for Pathogenic Neisseria

INTRODUCTION

The probability of success in the isolation of pathogenic neisseria from clinical specimens is related to three factors:

(i) the amount of care taken in obtaining good specimens and inoculating them correctly.

(ii) the provision of a culture medium capable of growing demanding strains of neisseria from small inocula.

(iii) the inclusion of selective agents in the medium which are capable of preventing overgrowth of commensal organisms on the enriched medium but which will not inhibit the growth of the species required.

The formulation of Thayer-Martin medium, as described, covers the second and third factors.

'Transgrow' medium, further described within, was formulated to include all the virtues of Thayer-Martin but with an increased agar concentration for the preparation of stable agar slants for transporting cultures in a carbon dioxide atmosphere.

Pure cultures of neisseria or strains sensitive to the selective antibiotics may be grown on 'chocolate' agar which contains heated blood as the sole enrichment factor.

The section concludes with advice on the important first factor; the techniques of collecting and inoculating specimens for the successful isolation of pathogenic neisseria.

DIRECTIONS

Thayer-Martin Medium

Thayer-Martin medium is prepared by suspending 18 g of Oxoid G.C. Agar Base in 200 ml of distilled water. Boil to dissolve the agar and sterilize by autoclaving at 121° C for 15 minutes.

Prepare a 2% w/v solution of Soluble Haemoglobin Powder (Oxoid L53) by adding 250 ml of **warm distilled water** to 5 g of haemoglobin powder. Continually stir the solution during the addition of water. Sterilize the solution by autoclaving at 121° C for 15 minutes.

Dissolve the contents of a vial of Sterile G.C. Supplement (Oxoid SR56) in 50 ml of sterile distilled water in a wide-necked flask.

Aseptically add the Supplement solution to the 200 ml of sterilized G.C. Agar Base, cooled to 50° C and mix gently. Add the 250 ml of sterilized Haemoglobin solution, cooled to 50° C, to the G.C. Agar Base-Supplement solution. Mix by swirling the medium to avoid trapping air-bubbles in the agar and pour plates.

'Transgrow' Medium

'Transgrow' medium is prepared by suspending 18 g of G.C. Agar Base and 5 g of Agar No. 1 (Oxoid L11) in 200 ml of distilled water. Boil to dissolve the agar and sterilize by autoclaving at 121° C for 15 minutes.

Prepare a 2% w/v solution of Soluble Haemoglobin Powder (Oxoid L53) by adding 250 ml of **warm distilled water** to 5 g of haemoglobin powder. Continually stir the solution during the addition of water. Sterilize the solution by autoclaving at 121° C for 15 minutes.

Dissolve the contents of a vial of Sterile G.C. Supplement (Oxoid SR56) in 50 ml of sterile distilled water in a wide-necked flask.

Aseptically add the Supplement solution to the 200 ml of sterilized G.C. Agar Base, cooled to 50° C and mix gently. Add the 250 ml of sterilized Haemoglobin solution, cooled to 50° C, to the G.C. Agar Base Supplement solution. Mix by swirling the medium to avoid trapping air-bubbles in the agar.

Dispense 8 ml of molten sterile 'Transgrow' medium into a horizontally positioned 1 oz. glass bottle. The rubber-lined screw-cap is loosely applied to the bottle and the medium is allowed to solidify in the horizontal position. A carbon dioxide atmosphere is introduced into the bottle by placing a group of bottles, containing solidified medium, upright in a vacuum jar. The air in the jar is partially exhausted by a vacuum pump and the chamber is refilled with a 10% CO_2/90% air mixture. This procedure should be repeated to ensure that the atmosphere of the chamber contains 10% CO_2. The caps of the bottles are then tightly fastened.

Non-selective Media

Both the above media can be prepared in a non-selective form by using Oxoid G.C. Supplement (without antibiotics, SR62) in place of the complete G.C. Supplement SR56. The G.C. Supplement without antibiotics is also useful where it is desired to add different antibiotics from those used in the complete supplement SR56.

'Chocolate' Agar

'Chocolate' Agar Medium is prepared by suspending 36 g of G.C. Agar Base in 1 litre of distilled water. Boil to dissolve the agar and sterilize at 121° C for 15 minutes.

Cool to 50° C and add 5–10% v/v defibrinated horse or rabbit blood and mix thoroughly without trapping air-bubbles.

Hold the medium at 80° C, mixing occasionally until it becomes a chocolate brown colour.

DESCRIPTION

Oxoid G.C. Agar Base (CM367) has been formulated to include Special Peptone (Oxoid L72) which is a mixture of meat and plant enzymatic digest. The presence of starch ensures that toxic products produced by the neisseria during growth are absorbed. Phosphate buffers are included to prevent amine production causing changes in the pH that would affect the survival of the organism in the medium.

Thayer & Martin (1966) described a medium in which an autoclaved haemoglobin solution replaced heated blood and the following antibiotics were included: Vancomycin 3μg/ml, Colistin Sulphonate 7.5μg/ml, Nystatin 12.5μg/ml. Trimethoprim lactate was shown by Seth (1970), Riddell & Buck (1970), Odegaard (1970) and Phillips (personal communication) to be of value in preventing *Proteus* species swarming and this antibiotic is now added at 5μg/ml to the other antibiotics (Martin & Lester, 1971). A sterile yeast concentrate was added to the medium as an enrichment supplement.

Martin & Lester (1971) modified Thayer & Martin medium by increasing the agar content and by the addition of dextrose (6 ml of a 25% w/v solution per litre of medium). The resulting medium called 'Transgrow', was prepared in screw-capped glass bottles as agar slopes which, after gassing with CO_2, could be used as a selective transport medium.

Oxoid G.C. Agar Base may be used in any of the formulations described by Thayer & Martin (1966) and Martin & Lester (1971).

The Oxoid G.C. Supplement Vials are pre-sterilized and contain the appropriate growth factors and antibiotics described by Thayer & Martin (1966) and Martin & Lester (1971) and they also contain Trimethoprim as described above. Thus the supplementation of the basal-haemoglobin-agar is convenient, economical and accurate.

ISOLATION TECHNIQUES

'Transgrow' is a selective medium for the transport and cultivation of *Neisseria gonorrhoeae* and is used for sending specimens to the Central Laboratories. Thayer-Martin plates are used when there is immediate access to a laboratory and this medium is the selective medium of choice for the isolation of *N. gonorrhoeae* and *N. meningitidis* from specimens taken from the throat, vagina, cervix, rectum or urethra, which contain mixed bacterial flora. Culture specimens may be obtained from the following sites in females:

(i) cervical cultures—after removal of the cervical mucus.

(ii) rectal cultures from the anal canal—site most likely to be positive when the cervical culture is negative.

(iii) urethral or vaginal cultures—which may be taken when cervical culture is unsatisfactory, e.g. hysterectomy patients and children.

In males the urethra culture is most commonly used, in which a sterile wire loop is used to obtain specimens from the urethra by gently scraping the mucosa. In homosexual males, additional culture specimens should be taken from rectum and pharynx.

To diagnose gonorrhoeae in women, culture specimens should be inoculated on to Thayer-Martin plates or 'Transgrow' bottles. The combination of a positive oxidase reaction of the colonies and microscopical demonstration of Gram negative diplococci, grown on either medium provides sufficient criteria for the diagnosis of gonorrhoeae.

In male patients, the microscopical demonstration of Gram-negative intracellular diplococci in a smear of urethral exudate, constitutes sufficient basis for a diagnosis of gonorrhoeae. When Gram-negative diplococci cannot be demonstrated by direct smear, specimens obtained from the anterior urethra should be inoculated on Thayer-Martin or 'Transgrow' medium. Oxidase positive colonies and typical microscopical morphology provide the criteria for a diagnosis of gonorrhoeae.

In special social, medico-legal and research situations when specific identification of organisms isolated on either Thayer-Martin or 'Transgrow' medium, from the pharyngeal or ano-genital region is desired, fermentation reactions should be used to confirm the identification of *N. gonorrhoeae.*

Fluorescent antibody staining can be used as a confirmatory tool for organisms isolated on either medium if insufficient isolated colonies of suspected gonococci are available for inoculation of the fermentation medium or for organisms that are no longer viable ('Criteria and Techniques for the Diagnosis of Gonorrhoeae' C.D.C. Venereal Diseases Branch, Atlanta, Georgia).

Smears may be prepared by rolling culture swabs on the surface of a glass slide: do not rub the swab on the slide because microscopic morphology will be distorted.

Thayer-Martin plates are inoculated by rolling the culture swab across a segment of the plate or preferably in a large 'Z' pattern so that an adequate area of the plate is inoculated. Streaking of the plate is carried out with a sterile wire loop to ensure adequate dispersion of the organisms. The plates should be incubated in a humid atmosphere containing 5–10% carbon dioxide at 35° C. Pathogenic neisseria form non-pigmented, translucent colonies. The antibiotics present in the medium inhibit practically all commensal organisms present in the specimen, including the saprophytic *Neisseria* species. *Mima polymorpha* var. *oxidans.* an organism that is sometimes confused with *Neisseria gonorrhoeae* is greatly inhibited on this medium.

'Transgrow' bottles are inoculated in the following manner:
holding the bottle upright to minimise loss of carbon dioxide, remove the cap, insert the specimen swab and allow it to soak up the condensate fluid in the bottle. Roll the swab from side to side across the medium, starting at the bottom of the bottle. After inoculation replace the bottle cap immediately.

If possible incubate the 'Transgrow' bottle at 35° C in an upright position for 16–18 hours before transport. This prior incubation enables sufficient growth to take place to ensure survival in prolonged transport and the organisms may be ready for identification when the bottle arrives at the laboratory.

At the laboratory, pre-incubated bottles may be examined immediately for *N. gonorrhoeae;* other bottles will be incubated at 35° C for 24–48 hours and examined thereafter.

'Transgrow' medium under 10% carbon dioxide permits the growth of pathogenic neisseria and suppresses contaminating organisms in a similar fashion to Thayer-Martin. It maintains the viability of pathogenic neisseria for more than 48 hours at room temperature.

'Transgrow' medium, prepared as above, and using bottles with gas tight seals, has been kept successfully for at least three months at room temperature, but its shelf life may be extended by storing at 4° C.

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Gelatin

Code L8

Gelatin is a proteinous material used for the solidification of culture media and for the detection and differentiation of certain proteolytic bacteria. Oxoid Gelatin is a bacteriological grade which has been manufactured and selected specifically for use in culture media. It is readily soluble in water to give a clear solution, free from sulphite and preservatives. A satisfactory firm gel is obtained from a 15% solution, and media containing this proportion of gelatin will withstand short-term autoclaving for 15 minutes at 121°C without significant loss of gel strength. Media containing gelatin can be cleared by the addition of the beaten white of one egg to every 500 ml of medium, prior to filtration and sterilization.

Oxoid Nutrient Gelatin CM135a is a complete medium, containing Gelatin, and does not require clearing during preparation—see page 205.

Guinea-Pig Kidney Antigen

Code BR8

A 20% emulsion in physiological saline, preserved with 0.5% phenol, prepared from the pooled, carefully trimmed and washed kidneys obtained from healthy animals. Guinea-Pig Kidney Antigen is a laboratory tested product for use with Ox-Cell Antigen BR9 (see page 178) and a 2% suspension of sheep red cells in the Paul-Bunnell reaction for the diagnosis of infective mononucleosis. The sheep cells should be more than one day and less than seven days old. Barrett's technique for the performance of this absorption test (Barrett, 1941) has been modified, see Cruickshank (1960).

Store at 4° C.

REFERENCES

Barrett, A. M. (1941) *J. Hyg.* **41**, 330.

Cruickshank, R. (1960) 'Mackie and McCartney's Handbook of Bacteriology, 10th ed., Livingstone Ltd., Edinburgh and London, pp. 320–323.

Hanks Stock Solution

Code BR19

FORMULA

Part 'A'		
Potassium chloride	*grams per litre*	**0.4**
Sodium phosphate		**0.06**
Potassium dihydrogen phosphate		**0.06**
Sodium chloride		**4.358**
Phenol red		**q.s.**
Part 'B'		
Calcium chloride		**0.14**
Magnesium sulphate		**0.1**
Magnesium chloride		**0.1**
Dextrose		**1.0**
Sodium chloride		**3.642**

DESCRIPTION

A twin-pack dehydrated Hanks Solution for those who prefer to reconstitute from concentrated stock solutions. Use of two stock solutions avoids the precipitation which occurs when a concentrate of the complete solution is heated. Sterile Oxoid Solutions 'A' and 'B' keep indefinitely without precipitation.

The mixed and sterilized stock solutions are equivalent to the balanced salt solution formulated by Hanks (1949) for use in tissue culture procedures. The prepared solution, designed to equilibriate with air, is used as the basis of tissue culture media and diluents. Hanks Solution serves to maintain the pH and osmotic pressure, and also to provide an adequate concentration of essential inorganic ions (Paul, 1959).

Tomlinson and Mitchell (1956) employed a similar solution (with the addition of penicillin and streptomycin) in their simple method for the isolation of poliomyelitis virus, from faeces, in tissue cultures of HeLa cells. They employed the solution: for the growth of HeLa cells (with added horse and rabbit serum); as a maintenance medium (with added lactalbumin hydrolysate); and for the emulsification of faeces.

Hanks Stock Solution is available in 100 g units (comprising sachets 'A' and 'B') sufficient to make 1 litre of each concentrated stock solution, which together are equivalent to 10 litres of final Hanks Solution.

DIRECTIONS

Dissolve contents of sachet 'A' in 1 litre de-ionized water and sterilize by autoclaving for 15 minutes at 121° C. This makes stock solution 'A'.

Dissolve contents of sachet 'B' in 1 litre de-ionized water and sterilize by autoclaving for 15 minutes at 121° C. This makes stock solution 'B'.

Add 100 ml stock solution 'A' followed by 100 ml stock solution 'B' to 800 ml de-ionized water. Mix and sterilize by autoclaving for 15 minutes at 121° C. Cool to room temperature and aseptically add 25 ml of sterile buffered bicarbonate solution.

REFERENCES

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Hartley Digest Broth

Code PM1185

DESCRIPTION

Hartley Digest Broth is a long established general-purpose medium used for the cultivation of a wide range of bacteria. The medium is prepared under controlled conditions by the tryptic digestion of de-fatted fresh ox-heart, according to the method described by Cruickshank (1962). A special feature of the Oxoid product is that the amino-nitrogen content is standardized to 0.06–0.07% w/v (formol titration). Hartley Digest Broth is subjected to chemical and bacteriological control tests in the Oxoid Laboratories, including measurements of the recovery from small inocula of fastidious organisms such as Lancefield group A, G and C streptococci and *Streptococcus pneumoniae*. This product is available only in the prepared form, it is not available as a dehydrated culture medium.

The broth may be used for many bacteriological purposes such as the cultivation of blood samples, sterility testing, the production of diphtheria toxin, and in Monckton enrichment medium for the isolation of *Corynebacterium diphtheriae* (Monckton, 1947).

REFERENCES

Cruickshank, R. (1962) 'Mackie and McCartney's Handbook of Bacteriology' 10th ed., Livingstone Ltd., Edinburgh and London, pp. 192–193.

Monckton, J. C. (1947) 'A Rapid Method for the Isolation of the Diphtheria Bacillus, using Blood Agar Plates' *Bull. Inst. Med. Lab. Technol.* **13**(1), 2–5.

Hektoen Enteric Agar

Code CM419

FORMULA

	grams per litre
Proteose Peptone (Oxoid L46)	12.0
Yeast Extract Powder (Oxoid L21)	3.0
Lactose	12.0
Sucrose	12.0
Salicin	2.0
Bile Salts No. 3 (Oxoid L56)	9.0
Sodium chloride	5.0
Sodium thiosulphate	5.0
Ammonium ferric citrate	1.5
Acid fuchsin	0.1
Bromthymol blue	0.065
Agar No. 3 (Oxoid L13)	14.0

pH 7.5 (approx)

DIRECTIONS

Suspend 76 g of the medium in 1 litre of distilled water and soak for 10 minutes. Heat gently and allow to boil for a few seconds to dissolve the agar. Do not autoclave. Cool to 60° C and pour plates.

DESCRIPTION

Hektoen Enteric Agar is a differential, selective medium for the isolation of *Shigella* and *Salmonella* species from enteric pathological specimens. The medium was developed to grow *Shigella* spp. as readily as other pathogens, while inhibiting the normal intestinal flora and to facilitate presumptive colonial identification of enteric pathogens with some degree of confidence (Taylor & Schelhaut 1971).

The increased carbohydrate and peptone content and lower toxicity of the indicator system overcame the inhibiting effects of the medium, allowing good recovery of shigellae (King & Metzger 1968).

TECHNIQUE

Inoculate the medium with fresh faeces suspended in Ringers solution or inoculate directly with rectal swabs. Spread the inoculum to obtain well separated colonies. Incubate for 18–24 hours at 37° C. Further incubation will improve differentiation between shigellae and salmonellae.

COLONIAL CHARACTERISTICS

Shigella spp., Providencia—green moist, raised colonies
Salmonella spp.—blue green colonies with or without black centres
Pseudomonas spp.—green or brownish flat, irregular colonies
Commensal organisms—salmon-coloured colonies

REFERENCES

King, S. and Metzger, W. I. (1968) 'A new plating medium for the isolation of enteric pathogens' *Appl. Microbiol.* **16,** (4), 577–578.

Taylor, W. I. and Schelhaut, D. (1971) 'Isolation of Shigellae. VIII. Comparison of Xylose Lysine Deoxycholate Agar, Hektoen Enteric Agar, Salmonella-Shigella Agar and Eosin Methylene Blue Agar with stool specimens'. *Appl. Microbiol.* **21,** (1), 32–37.

Horse Serum

Code SR35

Sterile unheated horse serum for use in bacteriological culture media. Horse Serum SR35 is also suitable for use in the serum tube or germ tube method of Mackenzie (1962) for the identification of *Candida albicans:*

1. Apply this test to only those isolates which grow at 37° C and form rounded budding cells. If microscopy shows a predominance of elongated or thread-like cells in young cultures, the isolate is not *Candida albicans.*

2. Lightly inoculate 0.5 ml of Horse Serum contained in a Durham tube or 'Bijou' bottle. The glassware need not be sterile.

3. Incubate for 3 hours at 37° C.

4. Examine microscopically. *Candida albicans* shows filamentous outgrowths ('germ tubes') from the rounded yeast cells.

5. This test has a high degree of specificity for *Candida albicans* but all positives should be checked for chlamydospore formation and biochemical characteristics. See Corn Meal Agar page 98 and E.M.B. Agar page 127.

REFERENCE

Mackenzie, D. W. R. (1962) 'Serum Tube Identification of *Candida albicans*' *J. clin. Path.* 15(6), 563–565.

Hoyle Medium Base

Code CM83

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	10
Peptone (Oxoid L37)	10
Sodium chloride	5
Agar No. 3 (Oxoid L13)	15

pH 7.8 (approx.)

DIRECTIONS

Suspend 40 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 55° C and add 50 ml of Laked Blood (SR48) and 10 ml of 3.5% Potassium Tellurite solution (SR30), mix, and pour plates.

DESCRIPTION

Hoyle medium is the well known modification (Hoyle, 1941) of Neill's medium for the cultural isolation and differentiation of *Corynebacterium diphtheriae* types. Hoyle medium does not exert the inhibitory effect manifested by Neill's on some *mitis* types, but gives very rapid growth with all types of *Corynebacterium diphtheriae,* so that diagnosis is possible after 18 hours' incubation.

TECHNIQUE

This is a highly selective medium which is used in parallel with non-selective media such as Loeffler Serum Medium (PM200) and blood agar (e.g. Blood Agar Base CM55 with 10% of horse blood). Unlike the non-selective media, Hoyle medium should be inoculated by rubbing the throat swab (or other material) over the entire surface—spreading with a platinum loop is not necessary.

Normally incubation for 18 hours at 37° C is sufficient but, when a negative result is obtained, incubation for up to 72 hours may be advisable. Gentian violet stained films made from colonies picked straight off the medium, are satisfactory for *C. diphtheriae* morphology. For the demonstration of the characteristic morphology and staining reactions of *C. diphtheriae* by Neisser's or Albert's method, it is preferable to utilize colonies from Loeffler Medium (PM200). The toxigenicity of *C. diphtheriae* strains may be determined by the Elek (1948) method. Oxoid Proteose Peptone L46 is especially recommended for use in the Elek medium.

COLONIAL CHARACTERISTICS

It is best to examine with a binocular low-power microscope, the colonies being illuminated from above by daylight.

Type differentiation is good, and typical colonial appearances after 18 hours' incubation are as follows:

C. diphtheriae type *gravis*—Grey colonies, 1.5–2.5 mm diameter dull, matt surface. May be slightly umbonate and show indented margins. Colonies can be pushed along the surface of the medium.

C. diphtheriae type *mitis*—Grey colonies, 1.5–2.0 mm diameter with regular margins and shining surface. Variation in size common.

C. diphtheriae type *intermedius*—Grey colonies, 0.5–0.75 mm diameter with shining surface. Colonies are very uniform in size with darker centres.

C. pseudodiphtheriticum (*C. hofmannii*)—Usually rounded, white or greyish, 0.5–0.75 mm diameter. Colonies may be up to 1 mm diameter, when they are black in more heavily inoculated areas and white when well isolated.

C. xerosis—Black, shining colonies of variable size.

Streptococci—Minute black or brownish-black colonies.

Other organisms—may occasionally grow which resemble *C. diphtheriae* type *intermedius* but are larger, while sporing anaerobes may produce brownish mucoid colonies.

It should be noted that not all corynebacteria produce the typical colonies described above—so in all cases it is advisable to use Hoyle medium in conjunction with the additional media and tests mentioned above.

REFERENCES

Elek, S. D. (1948) 'The Recognition of Toxicogenic Bacterial Strains in vitro *Brit. Med. J.* 1, 493–496.

Hoyle, L. (1941) 'A Tellurite Blood-Agar Medium for the Rapid Diagnosis of Diphtheria.' *Lancet* 1, 175–176.

I.D. Agar for Immuno-Electrophoresis

Code BR27

Electrophoresis on agar gel makes exacting demands on the medium. Ordinary commercial agars are not suitable and even those which have been clarified for immuno-diffusion work, require prolonged washing in distilled water before they are suitable for immuno-electrophoresis.

This new specially purified agar has been developed to meet the increasing demand for an agar of low conductivity, virtually free from chloride and sulphate ions, to act as a suitable medium for immuno-electrophoresis. It requires no further treatment.

For full details of technique, please ask for the separate Oxoid leaflet.

I.M. Diagnostic Reagent Card

Code BR29

The Oxoid I.M. Diagnostic Reagent Card meets the needs of laboratories seeking a rapid and convenient test for the diagnosis of Infectious Mononucleosis, in which results are easily interpreted.

For full details of technique, please ask for the separate Oxoid leaflet.

Incubating Pads

See page 184.

Incubating Tins

See page 184.

Iron Sulphite Agar

Code—Powder CM79
Tablets CM80

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**10.0**
Sodium sulphite		**0.5**
Iron citrate		**0.5**
Agar No. 3 (Oxoid L13)		**12.0**

pH 7.1 (approx.)

DIRECTIONS

Powder: Suspend 23.0 g in 1 litre of distilled water and boil to dissolve completely. Sterilize by autoclaving for 15 minutes at 121° C. Mix well before pouring.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This medium is a modification of Cameron Sulphite Agar developed by the National Canners Association of America (Tanner, 1944).

It has been shown that the medium is improved by reducing the concentration of sodium sulphite: Beerens (1958) showed that some strains of *Clostridium sporogenes* would not tolerate 0.1% sulphite. This was confirmed by Mossel *et al.* (1959) who consequently used iron sulphite agar containing only 0.05% sulphite and obtained no apparent inhibition.

Attenborough and Scarr (1957) and Bufton (1959), whilst using Oxoid Iron Sulphite Agar for the enumeration of *Clostridium nigrificans*, obtained low counts which suggested that the concentration of sulphite was too inhibitory. The medium was accordingly tried at half-strength, and was found to give more satisfactory results.

These findings have been substantiated in the Oxoid Laboratories and therefore the concentration of sodium sulphite has now been reduced from 0.1% to 0.05%.

TECHNIQUE

Iron Sulphite Agar is particularly suitable for the detection of thermophilic anaerobic organisms causing sulphide spoilage in food. The medium should be dispensed in 10 ml amounts in tubes for deep-shake cultures, and inoculated whilst fluid at about 50° C. Allow to set and incubate at 55° C for thermophilic species. *Clostridium nigrificans*, the type species, produces distinct black spherical growths in the depth of the medium.

In the Attenborough and Scarr method, diluted samples of the sugar were filtered through Oxoid Membrane Filters which were then rolled up and placed in tubes containing enough melted Iron Sulphite Agar (at approximately 50° C) to cover them. The medium was allowed to solidify and the tubes were incubated at 55° C. After 48 hours the number of black colonies on the membrane was counted. This membrane filter technique is quicker than the standard method but of comparable accuracy, and permits the examination of a much larger sample.

REFERENCES

Attenborough, Sheila J. and Scarr, M. Pamela (1957) 'The Use of Membrane Filter Techniques for Control of Thermophilic Spores in the Sugar Industry *J. appl. Bact.* **20**(3), 460–466.

Beerens, H. (1958) 'The Survival of Clostridia in Meat Brines DSIR, Proc. 2nd Internat. Symp. Food Microbiol. 1957, HMSO, London, pp. 235–245.

Button, A. W. J. (1959) 'A Note on the Enumeration of Thermophilic Sulphate-Reducing Bacteria (*Clostridium nigrificans*)' *J. appl. Bact.* **22**(2), 278–280.

Mossel, D. A. A., Golstein Brouwers, G. W. M.v. and de Bruin, A. S. (1959) 'A Simplified Method for the Isolation and Study of Obligate Anaerobes' *J. Path. Bact.* **78**(1), 290–291.

Scarr, M. Pamela (1959) 'Selective Media used in the Microbiological Examination of Sugar Products' *J. Sci. Food Agric.* **10**, 678–681.

Tanner, F. W. (1944) 'The Microbiology of Foods' 2nd ed., Garrard Press, Illinois, p. 1127.

'Iso-Sensitest Agar'

Code CM471

FORMULA

	grams per litre
Hydrolysed casein	**11.0**
Peptones	**3.0**
Dextrose	**2.0**
Sodium chloride (NaCl)	**3.0**
Soluble starch	**1.0**
di-Sodium hydrogen phosphate	**2.0**
Sodium acetate	**1.0**
Magnesium glycerophosphate	**0.2**
Calcium gluconate	**0.1**
Cobaltous sulphate ($CoSO_4$)	**0.001**
Cupric sulphate ($CuSO_4$)	**0.001**
Zinc sulphate ($ZnSO_4$)	**0.001**
Ferrous sulphate ($FeSO_4$)	**0.001**
Manganous chloride ($MnCl_2$)	**0.002**
Menadione	**0.001**
Cyanocobalamin	**0.001**
L-cysteine hydrochloride	**0.02**
L-Tryptophan	**0.02**
Pyridoxine	**0.003**
Pantothenate	**0.003**
Nicotinamide	**0.003**
Biotin	**0.0003**
Riboflavin	**0.003**
Thiamine	**0.00004**
Adenine	**0.01**
Guanine	**0.01**
Xanthine	**0.01**
Uracil	**0.01**
Agar No. 1 (Oxoid L 11)	**8.0**

pH 7.4 (approx.)

DIRECTIONS

Suspend 31.4 g in 1 litre of distilled water and bring to the boil to dissolve the agar. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Oxoid 'Iso-Sensitest Agar' has been prepared as a reproducible, semi-defined medium which will support the growth of the great majority of micro-organisms without further supplementation. It has been designed to overcome the objections made about Mueller-Hinton media by various workers (Ericsson & Sherris 1971, Garrod & Waterworth 1971, Barth-Reller *et al.* 1974, Duncan 1974, Yourassowsky, Vanderlinden & Schoutens 1974).

These objections were:

(i) different M.I.C. values in the broth and agar versions of the medium

(ii) agar versions showing antagonistic effects towards tetracycline

(iii) high levels of sulphonamide and trimethoprim antagonists

(iv) poor reproducibility with different manufacturers' peptones

(v) poor growth-supporting ability for streptococci and variable growth rates with gram-positive organisms generally.

Some rare mutant strains which are totally dependent on high levels of thymine and thimidine for their growth will, however, not grow in Oxoid 'Iso-Sensitest Agar', which has these two compounds at very low levels, since they are the naturally occurring antagonists of co-trimoxazole. Care must be taken to recognize these strains. (Tanner & Bullin 1974, Thomas & Bond 1973, Barker, Healing & Hutchinson 1972).

Oxoid 'Iso-Sensitest Agar,' CM 471 has been developed from Oxoid Sensitest Agar. This medium has been used successfully in several centres throughout the world (Reynolds, Hamilton, Miller & Brumfitt 1974, Stewart, Anderson & Miller 1975).

Developed in parallel with Sensitest Agar was Isotonic Sensitest Agar CM 447 (Neussel & Linzenmeier 1973) which contained known amounts of free mineral ions at physiological levels. This medium also enjoyed some success but the inhibition of the reaction for gentamycin to Pseudomonas species by 'free' magnesium and calcium ions proved to be troublesome to some workers. Equally the presence of 'free' calcium ions impaired the clarity of zones around tetracycline discs. Nevertheless, the concept of a stabilized mineral content in culture media was proved to be important. The considerable literature published on the effect of these substances on antibiotic action demonstrates this. (Garrod & Waterworth 1969, Gilbert, Kutscher, Ireland, Barnett & Stanford 1971, Zimelis & Jackson 1973, Newton 1953, Davis Iannetta & Wedgwood 1971, Ericsson & Sherris 1971, Hameister & Wahlig 1971, Brenner & Sherris 1972, Traub 1970).

Oxoid 'Iso-Sensitest Agar' is a logical combination of the two media, where stability in mineral content is combined with the ability to obtain optimum antibiotic zones of inhibition.

Barth-Reller, Schoenknecht, Kenny & Sherris (1974) demonstrated the contribution of cations normally provided in media by ordinary agars. They cause considerable variation in mineral content between the agar and broth media, as well as being an unstable source of essential ingredients. Only by using an agar such as Oxoid Agar No. 1 (L 11), which is specially processed to remove the free anions and cations, can a stabilized mineral content be obtained.

The amino-nitrogen base of acid-hydrolysed casein and special peptones has been supplemented with defined growth factors. Careful preparation of the nutrients ensures that trimethoprim and sulphonamide antagonists are at very low levels. Therefore the addition of lysed horse erythrocytes to the medium is not required when carrying out sensitivity tests with these compounds.

'Iso-Sensitest Broth'

Code CM473

Oxoid 'Iso-Sensitest Broth' has been produced in parallel with 'Iso-Sensitest Agar'. The broth has an identical nutrient formulation to the agar, due to the use of the specially purified agar. Where studies on antibiotic sensitivities are to be made in both broth and agar, this will be found to be of particular value.

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Stewart, Sheila, M., Anderson, Isobel, M. E., Malcolm, Margaret G. G. (1975) 'Detection of tetracycline resistance in Streptococcus pneumoniae.' *J. clin. Path.* **28,** 195–197.

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Kligler Iron Agar

Code—Powder CM33
Tablets CM34

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	3.0
Yeast Extract (Oxoid L20)		3.0
Peptone (Oxoid L37)		20.0
Sodium chloride		5.0
Lactose		10.0
Dextrose		1.0
Ferric citrate		0.3
Sodium thiosulphate		0.3

Phenol red	0.05
Agar No. 3 (Oxoid L13)	12.0

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 55 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute into containers. Sterilize by autoclaving at 121° C for 15 minutes. Allow to set as slopes with 1 in. butts.

Tablets: Add 1 tablet to 10 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes. Allow to set as slopes with 1 in. butts.

DESCRIPTION

A differential medium for the identification of *Enterobacteriaceae* on a basis of double sugar fermentation and hydrogen sulphide production. Oxoid Kligler Iron Agar based on the original medium (Kligler, 1917 and 1918; Bailey and Lacey, 1927) combines the principles of Russell (1911) double sugar agar with ferric citrate as an indicator to detect hydrogen sulphide production. The medium is recommended for the identification of colonies picked off from plating media such as MacConkey Agar, Bismuth Sulphite Agar, or Desoxycholate Citrate Agar, etc.

TECHNIQUE

Smear the surface of a Kligler Iron Agar slope and stab the butt with a colony picked off one of the solid media. The following reactions are obtained with this medium after incubation for 18 to 48 hours:

Organism	*Butt*	*Slope*	H_2S
Aerobacter aerogenes	AG	A	−
Aerobacter cloacae	AG	A	−
Escherichia coli	AG	A	−
Proteus vulgaris	AG	NC	+
Proteus morganii	A or AG	NC	−
Shigella dysenteriae (Shiga)	A	NC	−
Shigella sonnei	A	NC	−
Salmonella typhosa	A	NC or ALK	+
Salmonella paratyphi A	AG	NC or ALK	−
Salmonella paratyphi B	AG	NC or ALK	+
Salmonella schottmuelleri	AG	NC or ALK	+
Salmonella typhimurium	AG	NC or ALK	+
Salmonella choleraesuis	AG	NC or ALK	−
Salmonella enteritidis	AG	NC or ALK	+
Salmonella pullorum	AG	NC or ALK	+

AG = acid (yellow) and gas formation
A = acid (yellow)
NC = no change
ALK = alkaline (red)
+ = hydrogen sulphide (black)
− = no hydrogen sulphide (no black)

Fermentation of dextrose, but not lactose, produces a yellow slope which changes to red (alkaline) due to reversion of the reaction under aerobic conditions; the butt remains yellow. A yellow butt and red slope indicate the fermentation of dextrose only whilst a yellow slope and yellow butt indicate the fermentation of lactose only.

REFERENCES

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Kohn Two-Tube Medium

Medium No. 1 *Code—Powder CM179*
Tablets CM180
Medium No. 2 *Code—Powder CM181*
Tablets CM182

Medium No. 1

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	2.0
Peptone (Oxoid L37)	15.0
Yeast Extract (Oxoid L20)	2.0
Dextrose	1.0
Mannitol	10.0
Phenol red	0.05
Agar No. 3 (Oxoid L13)	16.0

pH 7.2 (approx.)

DIRECTIONS

Powder: Suspend 46 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115° C for 15 minutes. Cool to 60° C and add 25 ml of sterile 40% w/v Urea solution (SR20) per litre. Mix well, and pour slants with a generous butt. Inoculate slant and butt.

Tablets: Add 1 tablet to 10 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 115° C for 15 minutes. Cool to 60° C and add 5 drops 40% Urea Solution (SR20). Mix well and slope with a generous butt.

Medium No. 2

FORMULA

	grams per litre
Peptone (Oxoid L37)	10.0
Tryptone (Oxoid L42)	10.0
Sucrose	10.0
Salicin	10.0
Sodium chloride	5.0
Sodium thiosulphate	0.016
Disodium phosphate	0.09
Bromo-thymol blue	0.02
Agar No. 1 (Oxoid L11)	3.0

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 48 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115° C for 15 minutes. Mix well, and distribute into final containers. Gel vertically.

Tablets: Add 1 tablet to 10 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 115° C for 15 minutes. Gel vertically.

TEST PAPERS

To detect indole and hydrogen sulphide production, the following two test papers are necessary:

For Indole Test

Strips of tough filter paper about 5 mm × 50 mm are impregnated with the following solution:

p-dimethylaminobenzaldehyde . . .	5 g
o-phosphoric acid	10 ml
methanol	50 ml

and dried at 50° C for a minimum period.

For H_2S Test

Similarly impregnate strips with saturated lead acetate solution and dry in an oven at 70° C.

METHOD

Medium No. 1

Inoculate from pure cultures or with single colonies picked off solid selective media. Employ a straight wire, not a loop, stabbing deeply into the butt and smearing the surface of the slope.

After 18 to 24 hours incubation at 37° C, the production of acid (under aerobic conditions on the slope and under anaerobic conditions in the butt) can be detected by a change in the colour of the indicator.

The indicator, phenol red, incorporated in the medium shows a gradual colour change from yellow at pH 6.8 to cerise at pH 8.4. Organisms capable of fermenting only dextrose show a yellow butt with or without gas formation and the slant remains unchanged (red). A yellow slant indicates the fermentation of mannitol, whilst a positive urease reaction (i.e. alkaline) is indicated by a deep cerise colour of the whole medium.

Medium No. 2

Inoculate with a single stab to about one-third of the depth of the medium, using a straight wire. Suspend the two test papers above the medium by bending and wedging them between the cotton-wool plug and the side of the test tube. Incubate at 37° C for 18 to 24 hours and examine for motility, H_2S production, sugar fermentation, and indole production. The indicator, bromo-thymol blue, incorporated in the medium shows a gradual colour change from blue green at pH 7.4, to yellow at pH 6.0.

Motility is shown by feathery or diffuse growth spreading from the line of inoculation, or by turbidity of the whole medium.

H_2S production is indicated by the blackening of the lower part of the lead acetate paper strip.

Fermentation of sucrose, or salicin, or both, is indicated by the colour change of the indicator to yellow.

Indole formation is indicated by the yellow test paper becoming pink to red in colour.

TYPICAL REACTION (after Gillies, 1956)

Identifying Patterns of Organisms by the Composite Media (18 hours' incubation at 37° C):

	Medium No. 1			*Medium No. 2*			
	Fermentation of		*Urease Production*	*Fermentation of Sucrose/ Salicin*	*Motility*	*Production of H_2S*	*Formation of Indole*
	Dextrose	*Mannitol*					
S. typhosa	A	A	–	–	+	+	–
Other salmonellae	AG	A	–	–	+	±	–
Sh. sonnei	A	A	–	–	–	–	–
Sh. flexneri	A	A	–	–	–	–	±
Sh. schmitzi	A		–	–	–	–	+
*Proteus*group	(–)	(–)	+	AG or –	+	±	±

Fermentation Tests:

AG Acid and gas A Acid only – No reaction

± Variable reaction (–) Apparent negative reaction, urease activity masks effects of fermentation

Other Tests:

+ Positive – Negative ± Variable reaction

Notes:

Composite media for the study of the biochemical reactions of the *Enterobacteriaceae* have been deservedly popular for many years. Since 1911, when Russell first introduced his double-sugar medium, there have been numerous modifications. All these variations arise from the endeavour to obtain the maximum amount of information from culture in a single tube of medium; this obviously has its limits. With this fact in mind, Kohn (1954) developed a technique employing two tubes of composite media, thus permitting the study of the most important cultural reactions necessary for the identification of the *Enterobacteriaceae*. Later, Gillies (1956) introduced some minor modifications to Kohn media. In the same paper, he evaluated the technique by the examination of 2,108 organisms of faecal origin by this method, compared with the conventional methods. Gillies summarizes his findings as follows:

> 'Selection of *Salmonella* and *Shigella* organisms before serological identification was made by Kohn's technique; the modified composite media used were evaluated by comparison with the conventional sets of sugar media.
>
> 2,108 organisms of faecal origin were examined, and by use of composite media were grouped as 226 *Salmonella*, 836 *Shigella* and 1,046 "non-pathogenic". The parallel use of the conventional method revealed 208 *Salmonella*, 836 *Shigella* and 1,064 non-pathogenic organisms.
>
> No organisms were wrongly identified as "non-pathogenic" by the composite media; 18, however (0.85% of the total examined and 1.69% of those selected for serological confirmation), were provisionally included as *Salmonella* but were found to be serologically negative and thus correctly classed as non-pathogenic. These results compare

favourably with those obtained by the conventional method of preliminary identification of *Salmonella* and *Shigella* groups, and the adoption of the composite media in routine selection of these organisms effects economies in time, labour and glassware.'

Since then, many other workers have testified to the excellence of Kohn two-tube technique.

Oxoid Kohn Two-Tube Medium incorporates most of the modifications suggested by Gillies.

REFERENCES

Gillies, R. R. (1956) 'An Evaluation of Two Composite Media for Preliminary Identification of *Shigella* and *Salmonella*' *J. clin. Path.* **9**(4), 368–371.

Kohn, J. (1954) 'A Two-Tube Technique for the Identification of Organisms of the *Enterobacteriaceae* Group' *J. Path. Bact.* **67**(1), 286–288.

Russell, F. F. (1911) 'The Isolation of Typhoid Bacilli from Urine and Feces with the Description of a New Double Sugar Tube Medium' *J. Med. Res.* **25**, 217–229.

Koser Citrate Medium

Code CM65

FORMULA

	grams per litre
Sodium ammonium phosphate	**1.5**
Potassium dihydrogen phosphate	**1.0**
Magnesium sulphate	**0.2**
Sodium citrate	**2.5**
Bromo-thymol blue	**0.016**

pH 6.8 (approx.)

DIRECTIONS

Add 5.2 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.

Use chemically clean screw-capped bottles, or tubes closed with Oxoid Test Tube Caps. Do not use cotton wool plugs; if charred they may contaminate the medium and initiate growth of E. coli.

DESCRIPTION

Koser Citrate Medium is a chemically defined medium for the differentiation of the *coli-aerogenes* group on the basis of citrate utilization. The only source of nitrogen is the sodium ammonium phosphate while the sole carbon source is sodium citrate. The medium is prepared from certified pure chemicals and each batch is controlled to ensure the absence of any extraneous source of carbon.

Wilson and Miles (1957) discuss at some length the identifying criteria for the coliform group of organisms and note that only 6.71% isolated from human or animal faeces will give a positive citrate reaction whereas nearly 90% of coliforms isolated from soil will utilize citrate as a sole source of carbon.

TECHNIQUE

The medium is incubated for 2 to 4 days at 37° C. *Escherichia coli* I and II, because of their inability to use the citrate carbon source, show no growth and, therefore, no colour change. Other members of the group, which are able to utilize citrate, show growth and change of colour from light green to blue.

REFERENCE

Wilson, G. S. and Miles, A. A. (1957) 'Topley and Wilson's Principles of Bacteriology and Immunity' 4th ed., Edward Arnold Ltd., London, vol. I, p. 760.

KRANEP Agar Base

Code CM441

FORMULA

	grams per litre
Nutrient Agar (Oxoid CM3)	**28.0**
Potassium thiocyanate	**25.5**
Sodium pyruvate	**8.2**
Mannitol	**5.1**
Lithium chloride	**5.1**
Sodium azide	**0.05**
'Actidione'	**0.041**

pH 6.8 (approx)

DIRECTIONS

Suspend 72 g in 900 ml distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving for 15 minutes at 121° C. Cool to 50° C and aseptically add 100 ml Egg Yolk Emulsion (Oxoid SR 47). Mix well before pouring.

DESCRIPTION

The final agar medium containing the above ingredients was labelled KRANEP agar in accordance with the initial letters of its main diagnostic, selective and stimulatory agents, i.e. Kalium-Rhodanid-Actidione-Natriumazid-Eigelb-Pyruvate (Sinell & Baumgart, 1967). It is a selective product based on a medium containing potassium thiocyanate and mannitol, developed by Skorkovsky (1962) with the later additions of sodium azide and actidione as selective agents (Sinell & Baumgart, 1965). These workers also included sodium pyruvate as a selective growth stimulant (Baird-Parker, 1962) and egg yolk as a diagnostic agent (Gillespie & Alder, 1952).

Sinell & Baumgart (1967) recommended KRANEP agar for the selective enumeration of the total staphylococcal count from foodstuffs, as it yielded higher counts of coagulase-negative strains than did the other media tested. It has been cited in the literature for the selective cultivation of coagulase-positive staphylococci from meat products (Sinell & Kusch, 1969 and Sinell, Kusch & Untermann, 1970).

The selectivity of this medium is due to the inclusion of actidione and sodium azide. Actidione (cycloheximide, Upjohn Ltd.) is an antibiotic which inhibits the growth of most moulds and yeasts. Its use facilitates a selective bacterial count from food samples. During research on the isolation of staphylococci, Appleman (1965) found that sodium azide was successful in inhibiting highly aerobic contaminants e.g. *Bacillus* species. Such organisms may interfere in the cultivation of staphylococci from foods.

The agar is also inhibitory to enterococci, sarcinae and some gram-negative organisms e.g. *Proteus vulgaris* and *P. mirabilis* (Baird-Parker 1962 and Crisley, Peeler & Angelotti 1965). An additional advantage is that pigment formation is more readily detected when tellurite is not present.

TECHNIQUE

1. Dry the surface of prepared plates.
2. With a glass spatula, spread from 0.1 to 1.0 ml of dilutions of food (macerated in 0.1% peptone water) over the surface of each well-dried plate.
3. Incubate at 37° C and examine after 24 and 48 hours.

COLONIAL APPEARANCES

Staphylococcus aureus After 24 hours, this organism forms gold, shiny, convex colonies 1.0 to 1.5 mm in diameter. After incubation for 48 hours or more, opalescent zones showing lecithinase activity can be seen around the colonies.

Non-pathogenic staphylococci After 24 hours, white, shiny, convex colonies will have grown with a diameter of 0.2 to 0.5 mm. Further incubation may increase colony size, but there will be no lecithinase activity.

REFERENCES

Appleman, M. D. (1963) 'Studies on metabolic inhibitors for the isolation of staphylococci'. *J. appl. Bact.* **26,** ii, Society for Applied Bacteriology: Meetings.

Baird-Parker, A. C. (1962) 'An improved diagnostic and selective medium for isolating coagulase-positive staphylococci'. *J. appl. Bact.* **25,** (1), 12–19.

Crisley, F. D. Peeler, J. T. and Angelotti R. (1965) 'Comparative evaluation of five selective and differential media for the detection and enumeration of coagulase-positive staphylococci in foods'. *J. appl. Microbiol.* **13,** 140–156.

Gillespie, W. A. and Alder, V. G. (1952) 'Production of opacity in egg yolk media by coagulase-positive staphylococci'. *J. Pathol. Bacteriol.* **64,** 187–200.

Sinell, H. J. and Baumgart, J. (1965) 'Selektivnahrboden zur Isolierung von Staphylokokken aus Lebensmitteln'. *Zent Bl. Bakt.,* I. Abt. Orig, **197,** 447–461.

Sinell, H. J. and Baumgart, J. (1967) 'Selektivnahrboden mit Eigelb zur Isolierung von pathogenen Staphylokokken aus Lebensmitteln'. *Zent Bl. Bakt.,* I. Abt. Orig. **204,** 248–264.

Sinell, H. J. and Kusch, D. (1969) 'Selektivzuchtung von Koagulase Positiven Staphylokokken aus Hackfleisch'. *Arch. Hyg.* (Berlin) **153,** (1), 56–66.

Sinell, H. J., Kusch, D. and Untermann F. (1970) 'Zum Vorkommen von Koagulase positiven Staphylokokken in Hackfleisch'. *Zent Bl. Veterinarmed.* Reihe. **17,** (3), 429–435.

Skorkovsky, B. (1963) 'Selektivnahrboden fur Staphylokokken'. *Zent Bl. Bakt'* I. Abt. Orig 558–560.

'Lab-Lemco' Agar

Code—Powder CM17
Tablets CM18

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	**3**
Peptone (Oxoid L37)	**5**
Agar No. 3 (Oxoid L13)	**15**

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 23 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C. for 15 minutes

DESCRIPTION

This medium is the solidified form of 'Lab-Lemco' Broth, similar to that used in the United States as Nutrient Extract Agar. *Proteus* strains grow well on the medium but do not form spreading colonies. It is recommended for general laboratory use for the cultivation of organisms not exacting in their food requirements, and may be used as a basal medium to which materials are added to give selective, differential or enriched media. If the medium is to be enriched with blood it should be made isotonic by the addition of 0.5% of sodium chloride, in order to prevent haemolysis.

'Lab-Lemco' Broth

Code CM15

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	**3**
Peptone (Oxoid L37)	**5**

pH 7.4 (approx.)

DIRECTIONS

Add 8 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This medium was developed for workers who require a broth similar to those referred to as Nutrient Broth or Nutrient Extract Broth in American literature. 'Lab-Lemco' Broth is recommended for general laboratory use for the cultivation of organisms that are not exacting in their food requirements. It may be enriched with carbohydrates, serum, etc. If the medium is to be enriched with blood it should be made isotonic by the addition of 0.5% of sodium chloride, in order to prevent haemolysis.

'Lab-Lemco' Powder

Code L29

A REFINED EXTRACT PRODUCT OF VERY LIGHT COLOUR, IN POWDER FORM.

This product represents the ultimate in sophisticated media preparation. Made from specially selected extracts of *very light colour,* adjusted to neutrality and dried to a fine powder, the product has enormous advantages over conventional meat extract.

Because of the special processes employed in the preparation of this material, it can be used in conjunction with the other refined ingredients to make up culture media which require no filtration whatsoever.

Being only slightly hygroscopic, this product is very easy to handle. Its use eliminates the troublesome procedures associated with the weighing of conventional meat extracts which have a paste-like consistency.

When preparing culture media with 'Lab-Lemco' Powder it is only necessary to use 80% of the weight of ordinary meat extracts. The resultant broth is light and clear with excellent growth promoting properties.

It has been found that nutrient media incorporating 'Lab-Lemco' Powder will enhance the growth of many organisms. This characteristic is readily demonstrated with streptococci, where growth and haemolysis are markedly improved.

Analysis:

Moisture	6.0% w/w
Ash	15.6% w/w
Sodium chloride	7.2% w/w
Phosphorous as P_2O_5 . . .	0.7% w/w
Total Nitrogen	12.0% w/w
Amino Nitrogen	1.1% w/w
Calcium	0.16% w/w
Magnesium	0.04% w/w
Copper	1 ppm
Iron	112 ppm

pH 7.0

Lab-Ready Media

An inexpensive range of quality culture media in small sterile ready-to-use units.

The media used in this range are prepared and tested to high standards, and all batches are checked bacteriologically. In addition to growth supporting tests, the media are also controlled for their performance in the appropriate biochemical tests.

The following culture media are now available in this new range, packed in boxes of 24 of any one type of medium:

Culture Medium	*Code*	*Cap Code*
Nutrient Broth	R1	NB
Nutrient Agar Slopes	R2	NA
Urea Agar Slopes	R3	Plain Cap
Peptone Water	R4	PW
Cooked Meat Medium	R20	Plain Cap
Stuart Transport Medium (Modified) .	R22	STM
Simmons Citrate Agar Slopes . . .	R25	SCA
M.R.V.P. Medium	R26	MRVP
Trichomonas Medium No. 2 . . .	R27	TR
Decarboxylase Broth	R30	DB
Decarboxylase Broth (Arginine) . .	R31	DBA
Decarboxylase Broth (Ornithine) . .	R32	DBO
Decarboxylase Broth (Lysine) . . .	R33	DBL
Malonate Phenylalanine Broth . .	R34	MB
Gluconate Broth	R36	Black Cap
Hartley Digest Broth	R37	Off-set Black Spot
Selenite Broth	R39	Plain Cap
Dextrose Peptone Water	R53	Green Cap
Sucrose Peptone Water	R57	Blue Cap
Maltose Peptone Water	R58	Blue & White
Lactose Peptone Water	R59	Red Cap
Adonitol Peptone Water	R68	Silver Cap
Mannitol Peptone Water	R69	Mauve Cap
Dulcitol Peptone Water	R70	Pink Cap
Salicin Peptone Water.	R72	Pink & White

Lactalbumin Hydrolysate

See page 210.

Lactic Acid 10%

Code SR21

Sterile solution, available in 1 ml and 5 ml ampoules, in boxes of ten.

Lactose Bacteriological

Code L70

A special grade for inclusion in microbiological media.

Lactose 10 %

Code SR12

Filter sterilised solution, available in 5 ml ampoules, in boxes of ten.

Lactose Broth

Code CM137

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**3**
Peptone (Oxoid L37)		**5**
Lactose		**5**

pH 6.9 (approx.)

DIRECTIONS

Add 13 g to 1 litre of distilled water Mix well and distribute into containers fitted with Durham tubes. Sterilize by autoclaving at 121/ C for 15 minutes.

DESCRIPTION

Lactose Broth is recommended for use in the performance or confirmation of the Presumptive Test for members of the coliform group in water, milk, etc., as specified in United States official methods (see references).

Tubes of lactose broth are inoculated with dilutions of the water, etc. under test, incubated at 35° C, and examined for gas formation after 24 and 48 hours. In the tenth edition of 'Standard Methods for the Examination of Water, etc.', members of the coliform group are defined as those aerobic and faculative anaerobic Gram-negative non-sporing bacilli which ferment lactose with gas formation within 48 hours at 35° C.

REFERENCES

American Public Health Association (1955) 'Standard Methods for the Examination of Water, Sewage, and Industrial Wastes' 10th ed., APHA Inc., New York, pp. 368 and 375.
American Public Health Association (1953) 'Standard Methods for the Examination of Dairy Products' 10th ed., APHA Inc., New York, pp. 142 and 143.
United States Public Health Service (1946) 'Drinking Water Standards' *Public Health Reports* **61**, 376.

Lactose Ricinoleate Broth

Code CM371

FORMULA

Peptone (Oxoid L34)	*grams per litre*	**5**
Lactose		**10**
Sodium Ricinoleate		**1**

pH 7.6 (approx.)

DIRECTIONS

Dissolve 16 grams in 1 litre of distilled water. Distribute in 150 mm by 16 mm test tubes containing Durham fermentation tubes. Sterilize at 115° C for 20 minutes.

DESCRIPTION

With chlorinated waters it is essential to confirm the presence of coliform organisms by sub-culture to Lactose Ricinoleate Broth or Brilliant Green Bile (2%) Broth for incubation at 37° C for 48 hours in order to exclude false positive reactions due to aerobic and anaerobic gas-producing spore-bearing organisms. Production of gas in these media within 48 hours can be taken as sufficient confirmation that coliform organisms are in fact present.

Lactose Ricinoleate Broth was investigated by the P.H.L.S. Standing Committee on the Bacteriological Examination of Water Supplies (1968) as a possible alternative to Brilliant Green Bile (2%) Broth for the confirmation of coliform organisms and Esch. coli in water. It was concluded that Lactose Ricinoleate Broth produced fewer false negative reactions than Brilliant Green Bile (2%) Broth as a confirmatory medium at both 37° C and 44° C.

Lactose Ricinoleate Broth was recommended as a preferable alternative to Brilliant Green Bile (2%) Broth as it is not subject to the variability of Brilliant Green and Ox-bile.

The following table illustrates the results obtained in the trials:

Comparison of Lactose Ricinoleate Broth and Brilliant Green Bile (2%) Broth as confirmatory media for coliform organisms and E. coli.

Medium	*False* + ve	*False* − ve	*Total False*	*No. of strains tested*
	Tests at 37° C			
L.R. . .	42	91	133	2447
B.G.B. .	12	139	151	2447
	Tests at 44° C			
L.R. . .	16	50	66	2822
B.G.B. .	8	151	159	2822

The results show that Lactose Ricinoleate Broth was significantly better (P—0.01) than Brilliant Green Bile (2%) Broth at both 37° C and 44° C.

The greater number of false presumptive positive reactions shown by Lactose Ricinoleate Broth is an error in the right direction since in water bacteriology the most important point is not to miss any true coliform reactions. However the number of presumptive positive tubes, in which either medium gave a false result, was small.

The medium and method are fully described in The Bacteriological Examination of Water (Report 71, 1969).

TECHNIQUE

Lactose Ricinoleate Broth should be inoculated from the presumptive positive tube and incubated at 44° C for 24 hours. At the same time a tube of Tryptone Water (CM87) should be inoculated from the same presumptive positive tube and incubated at 44° C for 24 hours.

The production of gas from lactose at 44° C and the production of indole from the Tryptone Water at 44° C are accepted in the United Kingdom as evidence of Esch. coli. (P.H.L.S. Report 1968).

REFERENCES

P.H.L.S. Standing Committee on the Bacteriological Examination of Water Supplies (1969) 'A Minerals-Modified Glutamate Medium for the enumeration of coliform organisms in water' *J. Hyg.*, Camb. **67**, 367–374.

Report 71 (1969) 'The Bacteriological Examination of Water Supplies' 4th ed., London, HMSO.

Laked Horse Blood

See page 65.

Lauryl Tryptose Broth

(LAURYL SULPHATE BROTH)

Code–Powder CM 451
Tablets CM 452

FORMULA

Tryptose (Oxoid L47)	*grams per litre*	**20.0**
Lactose		**5.0**
Sodium chloride		**5.0**
di-Potassium hydrogen phosphate		**2.75**
Potassium dihydrogen phosphate		**2.75**
Sodium lauryl sulphate		**0.1**

pH 6.8 ± 0.2

DIRECTIONS

Powder: Dissolve 35.6 g in 1 litre of distilled water. Distribute into final containers and insert Durham tubes. Sterilize by autoclaving for 15 minutes at 121° C.

Tablets: Add 1 tablet to 10 ml of distilled water. Insert a Durham tube and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

The formulation of Lauryl Sulphate Broth tablets, (CM 144) has been amended to conform with the requirements set out in the Standard Methods for the Examination of Water and Wastewater by the American Public Health Association (1971).

The new formula, which incorporates Tryptose instead of Tryptone, provides a selective medium which is used for the detection of coliform organisms in water, dairy products and other foods. The A.P.H.A. recommend that Lauryl Tryptose Broth should be used for the Mean Probable Number Presumptive Test of coliforms in waters, effluent or sewage (A.P.H.A. 1971); as a confirmatory test of lactose fermentation with gas production for milk samples (A.P.H.A., 1967); and for the detection of coliforms in foods. (A.P.H.A., 1958).

Surface active agents have long been used as the inhibitory ingredients in selective media-MacConkey (1908) introduced bile salts for this purpose and later Albus and Holm (1926) working with lactobacilli found that sodium ricinoleate exerted a selective action. The development of synthetic wetting agents opened up new fields of investigation: Mallmann and Darby (1941), after comparative tests with a large number of these compounds, showed that sodium lauryl sulphate gave the best results in selective media for the coliform group.

Lauryl Tryptose Broth was designed to promote a rich growth and copious gas production from small inocula of coliform organisms. Aerobic sporing bacteria are completely inhibited. The advantage in using this product is that in addition to giving the fermentation reaction typical of Mac-Conkey Broth it can also be directly tested for the presence of indole. Unlike MacConkey Broth, the medium contains no indicator, but this can be added (if required) after incubation.

TECHNIQUE

For details of the A.P.H.A. standard methods please consult the references below.

Lauyrl Tryptose Broth is recommended for the detection and enumeration of coliform organisms in water and milk products, especially in the control of ice-cream manufacture and in dairy hygiene. A suggested procedure (Dyett, 1957) is as follows:

Inoculate samples of ice-cream into tubes of Lauryl Tryptose Broth in the matter normally employed in the MacConkey test (see page 177). Examine the tubes after overnight incubation at 37° C and, if no gas is visible, examine again at the end of 48 hours' incubation. For every tube showing fermentation ('primary fermentation') two further tubes of Lauryl Tryptose Broth are inoculated from a tube of the primary fermenting broth, and these are incubated at 37° C and 44° C respectively. It is advisable that the tube to be incubated at 44° C be warmed up in a water bath at this temperature before inoculation.

If the 44° C incubated broth ferments after seven hours, test for indole production with either Ehrlich or Kovacs reagent (see page 274). Due to the lauryl sulphate present, shaking the reagent culture mixture forms a persistent emulsion which interferes with the test. This may be avoided by shaking with ether, which separates rapidly, and then adding Kovacs reagent to the layer without shaking. If fermentation has not occurred after seven hours, leave the tube overnight at 44° C and test the following day. A positive indole reaction in a broth that has produced gas at 44° C indicates the presence of *Escherichia coli.* The tube at 37° C is incubated for 24 hours—if no fermentation occurs, the primary fermentation is assumed to be due to organisms other than coliforms. False positives are not uncommon in the primary fermentation tubes, due to fermentation of the sucrose in the added ice-cream by organisms other than coliforms.

After the two tubes of Lauryl Tryptose Broth have been inoculated for secondary fermentation, test the original primary fermentation tube (which was inoculated directly with ice-cream) for indole production. A positive reaction suggests the presence of E. coli and if this is indeed so, confirmation will be obtained later with the secondary fermentation from the 44° C bath. A negative indole reaction in the primary fermentation tube indicates the absence of E. coli.

REFERENCES

Albus, W. R. and Holm, G. E. (1926) 'The effect of Surface Tension upon the growth of *Lactobacillus acidophilus* and *Lactobacillus bulgarious' J. Bact.* **12,** 13–18.

A.P.H.A. (1966) 'Recommended Methods for the Microbiological Examination of Foods'. *2nd ed. Scharf, J.M.* (ed). New York.

A.P.H.A. (1967) 'Standard Methods for the Examination of Dairy Products'. *12th ed. Walter, W.G.* (ed). New York.

A.P.H.A. (1971) 'Standard Methods for the Examination of Water and Waste-water'. *13th ed.* p. 651, 664–665.

Dyett, E. J. (1957) 'Lauryl Sulphate Broth for the Testing of Ice-cream for E. coli.' *Lab. Prac.* **6,** (6), 327–328.

MacConkey, A. T. (1908) 'Bile Salt Media and their Advantages in some Bacteriological Examinations' *J. Hyg.* **8,** 322–334.

Mallmann, W. L. and Darby, C. W. (1941) 'Uses of a Lauryl Sulfate Tryptose Broth for the Detection of Coliform Organisms' *Am. J. Pub. Hlth,* **31,** 127–134.

Liver Agar (SHAPTON)

Tablets—Code CM292

FORMULA

Infusion from fresh liver	*grams per litre*	**23.0**
Peptone (Oxoid L37)		**10.0**
di-Potassium phosphate		**1.0**
Liver fibre		**47.0**
Agar No. 3 (Oxoid L13)		**15.0**

pH 6.9 (approx)

DIRECTIONS

Add one tablet to 5 ml of distilled water. Soak for 15 minutes and sterilize by autoclaving for 20 minutes at 10 lb per square inch (115° C). (See section on Technique).

DESCRIPTION

Liver Agar (Shapton) is a new medium for the screening of food materials for the spores of thermophilic gas-producing anaerobes. The medium, which was originally formulated by Shapton, has been employed for several years in the laboratories of H. J. Heinz and Company Limited. This medium may be used in conjunction with Shapton Medium (CM270) after the heat treatment described by Shapton and Hindes (1963).

TECHNIQUE

A suggested technique for the use of Liver Agar (Shapton) is as follows:

1. Prepare a 20% w/w suspension of the food sample to be tested in ¼-strength Ringer solution (Oxoid BR52).

2. Place three tablets of Liver Agar (CM292) and 15 ml of distilled water in each of six 6 × ⅝ in. test tubes and soak for 15 minutes Loosely insert rubber seals (Astell, Brevete, etc.) and sterilize by autoclaving for 20 minutes at 10 lb per square inch (115° C).
3. Remove the 6 tubes from the autoclave and inoculate them with a total of 20 ml of food suspension (i.e. an average of 3⅓ ml per tube). Do not allow the tubes of medium to cool below 80° C before inoculation.
4. Press home the rubber seals and immediately place the tubes of inoculated medium in a pre-heated autoclave. Close the lid of the autoclave and continue heating with the vent valve open until the autoclave temperature reaches 100° C and steam issues freely. Close the vent, allow the temperature to rise to 108.3° C (227° F or 5 lb per square inch), and maintain this temperature and pressure for 10 minutes.

   Turn off the heat source and allow the autoclave temperature to fall naturally to below 99° C. Open the vent valve and the lid, and remove the tubes. Push home the rubber seals and allow the tubes of medium to set for approximately 30 minutes.
5. Incubate for 72 hours at 55° C.
6. Examine for splitting of the medium by gas production. In Shapton's view (1965), the original food sample contains an excess of thermophilic gas-producing anaerobes if more than 4 out of the 6 tubes show splitting of the medium.

N.B. For results which are to be reproducible between laboratories it is important to use autoclaves with similar thermal characteristics.

REFERENCES

Shapton, D. A. and Hindes, W. R. (1963) 'The Standardisation of a Spore Count Technique *Chemistry and Industry,* February 9th, p. 230–234.

Shapton, D. A. (1965) Personal Communication.

Liver Broth

Code—Granules CM77
Tablets CM78

FORMULA

Infusion from fresh liver	*grams per litre*	**23**
Peptone (Oxoid L37)		**10**
Potassium phosphate		**1**
Extracted liver tissue		**30**

pH 6.8 (approx.)

DIRECTIONS

Granules: Add 64 grams to 1 litre of distilled water and soak for 15 minutes with occasional stirring. Distribute into 18 mm (¾ in.) diameter tubes to a depth of 50 mm (2 in.) so that the bottom of the tube is filled with liver particles. Agitate frequently during distribution to keep the liver tissue in suspension. Sterilize by autoclaving for 20 minutes at 115° C. Inoculate when cool and then aseptically seal with a layer of sterile 2% Oxoid Agar No. 3 solution.

Tablets: Add 1 tablet to 10 ml of distilled water in an 18 mm (¾ in.) diameter tube and soak for 5 minutes. Sterilize by autoclaving for 20 minutes at 115° C. Each tablet contains sufficient liver particles to cover the bottom of an 18 mm (¾ in.) diameter test tube. Inoculate when cool and then aseptically seal with a layer of sterile 2% Oxoid Agar No. 3 solution. TABLETS ARE PARTICULARLY CONVENIENT BECAUSE THEY ENSURE EVEN DISTRIBUTION OF THE PARTICULATE LIVER.

DESCRIPTION

A liver infusion medium, containing liver particles, for the examination of foods for saccharolytic or putrefactive mesophilic and thermophilic anaerobes. Also recommended for the maintenance of aerobes and anaerobes in pure culture.

TECHNIQUE

Gillespie in communication with Scarr (1957) recommended Oxoid Liver Broth for the examination of canners' sugar for hydrogen swells caused by thermophilic anaerobes (*Clostridium thermosaccharolyticum*). A 20% w/v solution of the sugar is steamed for 30 minutes to destroy vegetative forms and inoculated into Oxoid Liver Broth sealed with agar. The standard proposed was a maximum of 1 positive tube in six—with 20 ml inocula incubated for 72 hours at 56° C.

This medium should be made up only when required for use. Storage of the reconstituted medium is not recommended because air may be absorbed and the re-steaming necessary for the restoration of anaerobic conditions darkens the medium. Liver broth is not an homogeneous medium, consisting of a layer of liver particles and a cloudy supernatant. Growth produces an obvious increase in turbidity and some organisms (e.g. *Cl. thermosaccharolyticum*) also produce gas which often pushes the agar plug towards the top of the tube. Some organisms also digest the solid liver tissue.

REFERENCE

Scarr, M. Pamela (1958) 'Bacteriology of White Sugar in Relation to the Curing and Packing of Meat' DSIR, Proc. 2nd Internat. Symp. Food Microbiol. 1957, H.M.S.O., London, pp. 29–33.

Liver Desiccated

Code L26

Dehydrated whole liver, specially manufactured for the preparation of infusion media. Liver Desiccated is prepared by the dehydration of fresh ox livers under carefully controlled conditions designed to ensure maximum retention of nutritive properties, and is equivalent to five times its weight of fresh liver.

To prepare a liver infusion medium, add 50 grams of Liver Desiccated to 1 litre of distilled water and allow to infuse (with frequent agitation) for 1 hour at 50° C. Boil the mixture for a few minutes to coagulate protein, strain through 60-mesh stainless steel gauze, add 10 grams of Peptone (Oxoid L37) and 5 grams of sodium chloride. Adjust the reaction to pH 7.2, boil, strain through gauze as above, and sterilize by autoclaving at 121° C for 15 minutes.

Liver Digest

See page 210.

Liver Infusion

Code L25

The well recognized value of liver extract for the enrichment of culture media is largely due to the presence of growth-stimulating substances. Manufactured from fresh ox livers by an infusion process designed to preserve these water-soluble nutrients, Oxoid Liver Infusion has been developed to meet the need for a bacteriological liver extract. It has been shown that filtration through fibrous material removes some of the essential nutrients from the infusion, so the product is not filtered at any stage of its preparation and consequently does not produce a clear solution. Media made with this extract should not be filtered but, if required, they may be clarified by centrifugation or by allowing them to settle and then decanting off supernatant fluid.

This infusion is an extremely potent source of bacterial growth factors, and is recommended for the preparation of all media where liver infusion is specified. Difficulties encountered in the use of liver infusion are frequently attributed to differences in the raw material, but use of the Oxoid product enables the technologist to eliminate variation.

Liver Infusion has also given excellent results when used to prepare the media described by Nymon and Gortner (1946) for the cultivation of lactobacilli.

In his paper on the isolation of *Mycobacterium paratuberculosis* (Johne's Bacillus), Minett (1942) recommended the inclusion of liver extract in the medium.

The use of liver infusion media for the growth of anaerobes has been normal practice for many years, and numerous formulae have been published. Liver Infusion will give excellent results in the preparation of liver broth (Cameron *et al.*) and other variations of anaerobic culture media, such as brain liver heart sloppy agar and liver veal agar.

Oxoid Liver Infusion is supplied in granular form. One gram dissolved in 100 ml distilled water will provide the equivalent of the infusion to be obtained from 25 grams of fresh liver in 100 ml of water.

For media requiring the addition of whole liver tissue, the use of Liver Desiccated L26 is recommended. See also, Liver Digest L27.

REFERENCES

Cameron, Williams and Thompson *et al.*, *Bull. Nat. Canners Assoc.*, No. 25.
Minett, F. C. (1942) *J. Path. Bact.* **54**, 214.
Nymon, Mavis C. and Gortner, W. A. (1946) *J. Biol. Chem.* **163**, 277–282.

Loeffler Serum Medium

Code PM200

FORMULA

Horse Serum (Oxoid SR35)		**750 ml**
'Lab-Lemco Powder (Oxoid L29)	*grams*	**2.5**
Peptone (Oxoid L37)		**2.5**
Dextrose		**2.5**
Sodium chloride		**1.25**

and distilled water to 1 litre

DESCRIPTION

Loeffler Serum Medium in sterile ready-to-use form for the cultivation of corynebacteria and other fastidious organisms, and also for the demonstration of proteolysis.

The Oxoid medium differs little from the original described by Loeffler (1887). Due to its high serum content Loeffler Serum Medium is suitable for the cultivation of corynebacteria and also for other organisms, where it is important that they should exhibit their natural morphological and physiological characteristics. Thus this medium is used to elicit the characteristic staining reaction of *C. diphtheriae* when stained by Neisser's method. Loeffler Serum Medium is also often employed for the primary or secondary cultivation of micro-organisms, so that they may display their natural virulence, microscopic and colonial morphology, and chromogenesis, as well as staining reactions. The medium will often restore these properties after they have been lost as a result of prolonged subcultivation on other media. Because of its serum content, the medium may also be used to determine the proteolytic activity of organisms.

TECHNIQUE

In the investigation for diphtheria, rub a swab over the surface of a Loeffler Serum Medium slope and also over the surface of a Hoyle Medium (Code CM83) plate. Incubate for 18 to 24 hours at 37° C. and prepare stained smears from the surface of the slope.

To determine the proteolytic activity of aerobes, incubate a Loeffler Serum Medium slope in the usual manner; to demonstrate proteolysis by anaerobes (usually *Clostridium* species) incubate anaerobically or fill the slope bottle with Brewer Thioglycollate Medium CM23 before incubation. Proteolytic colonies are initially surrounded by small craters of liquefied medium and may subsequently liquefy the slope with the production of a putrefactive odour. It is usually necessary to incubate Loeffler medium for 3–4 days, or much longer, before proteolysis is apparent (Willis, 1960).

REFERENCES

Loeffler, F. (1887) *Berliner militararztliche Gesellschaft Sitzing. Centr. f Bakt.* **2**, p. 105.
Willis, A. T. (1960) 'Anaerobic Bacteriology' Butterworth and Co., London.

Löwenstein-Jensen Medium

Code PMI

FORMULA

Potassium dihydrogen phosphate	*grams*	**2.4**
Magnesium sulphate		**0.24**
Magnesium citrate		**0.6**
Asparagine		**3.6**
Glycerol		**12.0 ml**
Potato starch		**30.0**
Fresh egg mixture (yolks and whites)		**1,000 ml**
Malachite green		**0.4**

and water to 1,640 ml

DESCRIPTION

An inspissated egg medium for the cultivation and differentiation of *Mycobacterium* species, especially *M. tuberculosis* (*M. tuberculosis* var. *hominis*) and *M. bovis* (*M. tuberculosis* var. *bovis*). Growth of *M. tuberculosis* is enhanced by the incorporation of glycerol, whilst the growth of *M. bovis* and certain other mycobacteria is not enhanced and may even be inhibited. The Oxoid medium corresponds to the modification of the Löwenstein (1931) medium developed by Jensen (1932).

TECHNIQUE

The specimen is inoculated directly on to the surface of the medium; material which is contaminated with other micro-organisms, or which contains few mycobacteria, may be subjected to one of the numerous concentration methods before culturing (e.g., see page 54). Löwenstein-Jensen Medium may also be used in conjunction with Oxoid Membrane Filters (see page 155). It is preferable to inoculate duplicate slopes of medium with each specimen. Incubate at 37° C and examine after 10 to 14 days, and then every week until the culture is discarded after a total of 8 weeks incubation. *Mycobacterium tuberculosis* grows well to produce large dry, heaped-up yellowish colonies, whilst *M. bovis* fails to grow or is only able to produce small, 'colourless', discrete colonies. For the cultivation of glycerol sensitive mycobacteria, such as some strains of *M. bovis*, this medium is available as Löwenstein-Jensen Medium (without glycerol) Code Number PM2.

REFERENCES

Jensen, K. A. (1932) 'Reinzüchtung und Typen bestimmung von Tuberkelbazillenstammen *Zent. f. Bakt., Parasitenk. u. Infektionskr.* I. Abt. Orig., **125**, 222–239.
Löwenstein, E. (1931) 'Die Zuchtung der Tuberkelbazillen aus dem strömenden Blute *Zent. f. Bakt., Parasitenk. u. Infektionskr.* I. Abt. Orig., **120**, 127–129.

Lysine Decarboxylase Broth

(TAYLOR MODIFICATION)

Code CM308

FORMULA

Yeast Extract (Oxoid L21)	*grams per litre*	**3.0**
Dextrose		**1.0**
L-Lysine		**5.0**
Brom-cresol purple		**0.016**

pH 6.1 (approx.)

DIRECTIONS

Add 1 tablet to 5 ml of distilled water in a ¼ oz screw-capped bottle. Sterilize by autoclaving at 121° C. for 15 minutes.

DESCRIPTION

Lysine Decarboxylase Broth is a diagnostic medium recommended for distinguishing salmonellae and Arizona organisms from the Bethesda-Ballerup group of Enterobacteriaceae.

Taylor's modification of the medium (1961) shows an improved performance over the formulation described by Falkow (1958). This is achieved by omitting peptone from the medium, thus eliminating false positives caused by *Citrobacter freundii* and its paracolon types. These organisms utilize peptone as a nitrogen source, produce an alkaline reaction and mask the absence of lysine decarboxylase.

Taylor's modification shares the advantages of Falkow's formulation over that of Moller (1955) in that it does not require the special conditions of anaerobic culture and low pH and it is relatively easy to control.

During the initial stages of incubation, fermentation of dextrose by the organism, with production of acid, results in a colour change in the indicator to yellow. On further incubation, if lysine is decarboxylated to cadaverine, there will be an alkaline reaction. The indicator colour will then change to purple (positive). If the colour remains yellow, the reaction is negative.

Decarboxylase reactions of various members of the Enterobacteriaceae on Lysine

Organism	*Lysine decarboxylation*
Arizona	+
Bethesda-Ballerup	−
Salmonella typhi	+
Salmonella paratyphi A	−
Other types of Salmonellae	+
Shigella (pathogenic species)	−
Alkalescens-Dispar (most strains)	+
E. coli	±
Citrobacter	−
Providencia	−
Proteus	−
Klebsiella	+
Serratia	+

TECHNIQUE

The medium is inoculated with the organism and incubated for 24 hours at 37° C.

Results after 24 hours:

Purple colour—positive reaction
Yellow colour—negative reaction

REFERENCES

Falkow, S. (1958) 'Activity of lysine decarboxylase as an aid in the identification of Salmonella and Shigella' *Amer. J. Clin. Path.* **29,** 598–600.
Moller, V. (1955) 'Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system' *Acta path. Microbiol. Scand.* **56,** 158–172.
Taylor, W. I. (1961) 'Isolation of Salmonellae from Food Supplies. V. Determination of the Method of Choice for Enumeration of Salmonella' *Appl. Microbiol.* **9,** 487–490.

Lysine Iron Agar

Code CM381

FORMULA

	grams per litre
Bacteriological Peptone (Oxoid L34)	**5.0**
Yeast Extract (Oxoid L21)	**3.0**
Dextrose	**1.0**
L-Lysine	**10.0**
Ferric ammonium citrate	**0.5**
Sodium thiosulphate	**0.04**
Brom-cresol purple	**0.02**
Agar No. 3 (Oxoid L13)	**14.5**

pH 6.7 (approx.)

DIRECTIONS

Suspend 34 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense into tubes and sterilize by autoclaving at 121° C for 15 minutes. Cool the tubes in an inclined position to form slants with deep butts.

DESCRIPTION

Edwards & Fife (1961) described the formulation and use of Lysine Iron Agar to detect Arizona group organisms that could rapidly ferment lactose. These strains would produce red or pink colonies on Maconkey Agar or Desoxycholate Citrate Agar and yellow colonies on Brilliant Green Agar. In the usual examination for enteric pathogens, these organisms would be overlooked. Further, many of these cultures, when transferred to Triple Sugar Iron (T.S.I.) Agar slants, produced acid conditions in the medium so quickly that the expected positive reaction for hydrogen sulphide was suppressed. Since Arizona types which ferment lactose rapidly are found occasionally in outbreaks of food infection, it is important to determine their occurrence.

The only recognised groups of Enterobacteriaceae which regularly decarboxylate lysine rapidly and which produce large amounts of hydrogen sulphide, are members of the salmonella and Arizona groups (Moeller 1954 and Ewin, Davis & Edwards 1960).

Lysine Iron Agar is therefore a sensitive medium for the detection of both salmonella and Arizona organisms. The medium is tubed, sterilized and slanted so that a short slant and deep butt are formed. It is inoculated with a straight needle by stabbing to the base of the butt and streaking the slant. The caps of the tubes must be replaced loosely so that aerobic conditions prevail on the slant. Incubate at 37° C, overnight.

Cultures which rapidly produce lysine decarboxylase cause an alkaline reaction (purple colour) throughout the medium. Those organisms that do not decarboxylate lysine produce an alkaline slant and an acid butt (yellow colour).

Cultures which produce hydrogen sulphide cause an intense blackening in the medium.

Due to deamination of the lysine, *Proteus* and *Providence* cultures produce a red slant over an acid butt.

REACTIONS

Cultures	*Slant*	*Butt*	H_2S
Arizona	Alkaline	Alkaline	+
Salmonella	Alkaline	Alkaline	+
Proteus	Red	Acid	−
Providence	Red	Acid	−
Citrobacter	Alkaline	Acid	+
Escherichia	Alkaline	Acid or neutral	−
Shigella	Alkaline	Acid	−
Klebsiella	Alkaline	Alkaline	−

Thatcher & Clark (1968) described a procedure for the isolation of salmonellae from foods in which suspect colonies from selective agar plates were purified and then inoculated into Lysine Iron Agar and Triple Sugar Iron Agar. Using this combination of media a greater discrimination can be made between the coliform organisms, e.g. *Escherichia* and *Shigella.*

Timms (1971) described the techniques of isolation and identification of Arizona infection in turkeys, using Lysine Iron Agar.

REFERENCES

Edwards, P. R. and Fife, Mary A. (1961) 'Lysine-Iron Agar in the detection of Arizona cultures' *Appl. Microbiol.* **9,** 478–480.

Ewing, W. H., Davis, B. R. and Edwards, P. R. (1960) 'The decarboxylase reactions of Enterobacteriaceae and their value in taxonomy' *Pub. Hlth Labs.* **18,** 77–83.

Moeller, V. (1954) 'Distribution of amino-acid decarboxylases in Enterobacteriaceae' *Acta. Pathol. Microbiol. Scand.* **35,** 259–277.

Thatcher, F. S. and Clark, D. S. (1968) 'Micro-organisms in Food' *University of Toronto Press,* p. 100.

Timms, L. (1971) 'Arizona Infection in Turkeys in Great Britain *Med. Lab. Techn.* **28,** 150–156.

Lysine Medium

Code CM191

FORMULA

Dextrose	*grams per litre*	**44.5**
Potassium dihydrogen phosphate		**1.78**
Magnesium sulphate		**0.89**
Calcium chloride fused		**0.178**
Sodium chloride		**0.089**
Adenine		**0.00178**
DL-Methionine		**0.000891**
L-Histidine		**0.000891**
DL-Tryptophane		**0.000891**
Boric acid		**0.0000089**
Zinc sulphate		**0.0000356**
Ammonium molybdate		**0.0000178**
Manganese sulphate		**0.0000356**
Ferrous sulphate		**0.0002225**
Lysine		**1.0**
Inositol		**0.02**
Calcium pantothenate		**0.002**
Aneurine		**0.0004**
Pyridoxine		**0.0004**
p-Aminobenzoic acid		**0.0002**
Nicotinic acid		**0.0004**
Riboflavin		**0.0002**
Biotin		**0.000002**
Folic acid		**0.000001**
Agar No. 3 (Oxoid L13)		**17.8**

DIRECTIONS

Suspend 6.6 g in 100 ml distilled water containing 1.0 ml Potassium Lactate 50% SR37. Bring to the boil to dissolve completely. Agitate frequently to prevent superheating. Cool to 50° C and add 0.1 ml of Lactic Acid 10% SR21 to adjust to pH 5.0 (approx.). Dispense into Petri dishes and remove surface moisture by drying at 37° C.

DESCRIPTION

A complex medium, originally described by Morris & Eddy (1957) for the isolation and enumeration of wild yeasts in pitching yeast. Walters and Thiselton (1953) examined 180 species of yeasts in a liquid synthetic medium containing lysine as the sole nitrogen source. They found that no normal *cerevisiae* or *carlsbergensis* strains utilized lysine whereas many other yeasts, including wild yeasts, did so. They kept their stock cultures on malt extract agar slopes or on malt extract chalk agar in the case of *Brettanomyces* species. Later, Morris & Eddy (1957) described a solid lysine medium for the isolation and enumeration of wild yeasts in pitching yeast. Oxoid Lysine Agar is made to their published formula.

TECHNIQUE

Wash and centrifuge the sample of pitching yeast three times with distilled water. Remove 0.2 ml of a suspension containing approximately 10^7 cells per ml and spread with a bent platinum wire, over the surface of a Lysine Medium plate. Incubate at 25° C and examine daily for evidence of growth. Count the number of colonies which develop, and express the degree of contamination as the number of wild yeast cells per million cells of the original inoculum.

The number of cells in the inoculum is important as it has been shown by Morris & Eddy that small numbers of cells (approximately 100 to 1,000) still grow to a limited extent on the medium. Where the number of brewing yeast cells exceeds approximately 10,000, a count of the colonies developing provides a direct measure of the contamination by wild yeasts.

REFERENCES

Morris, E. O. and Eddy, A. A. (1957) 'Method for the Measurement of Wild Yeast Infection in Pitching Yeast' *J. Inst. Brew.* **63**(1), 34–35.

Walters, L. S. and Thiselton, M. R. (1953) *J Inst. Brew.* **59,** 401.

MacConkey Agar

Code—Powder CM7
Tablets CM8

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**20.0**
Lactose		**10.0**
Bile Salts (Oxoid L55)		**5.0**
Sodium chloride		**5.0**
Neutral red		**0.075**
Agar No. 3 (Oxoid L13)		**12.0**

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 52 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Dry the surface of the gel before inoculation.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A differential medium for the detection, isolation and enumeration of coliforms and intestinal pathogens in water, dairy products and biological specimens. Oxoid CM7 corresponds to the medium recommended by the World Health Organization (1963), the Dept. of Health (1969) and by Windle Taylor (1958) for the bacteriological examination of water.

Although principally used for coliforms, this medium may also be employed for the differentiation of other enteric bacteria (including pathogens) and is suitable for the differentiation of *Pasteurella* species (e.g. Hoogendijk, 1962).

TECHNIQUE

Pathological Specimens

Due to its ability to support the growth of pathogenic Gram-positive cocci (e.g. staphylococci and enterococci) as well as Enterobacteriaceae, MacConkey Agar CM7 is particularly recommended for the cultivation of pathogens which may be present in a variety of specimens such as urine, faeces and wound swabs. Whilst it is selective it does not suppress a mixed bacterial flora to the same extent as other inhibitory media (including other MacConkey agars). It provides a number of other diagnostic indications in addition to bile tolerance, such as colony morphology and chromogenesis. MacConkey Agar should be used in parallel with other selective indicator media such as Desoxycholate Citrate Agar, Bismuth Sulphite Agar, Brilliant Green Agar and Brilliant Green Bile (2%) Broth, and a non-selective medium such as Blood Agar.

Water Examination (Windle Taylor, 1958) (Dept. of Health, 1969)

The medium may be used for the direct count of *coli-aerogenes* bacteria, using pour-plates prepared from known volumes of the water sample, but a more exact role for the medium is for the differentiation of organisms producing acid and gas in MacConkey Broth at 37° C: all positive broth tubes are plated on MacConkey Agar, the plates are incubated for 24 hours at 37° C and examined for typical colonies (see below). Colonies composed of Gram-negative non-sporing rods are subcultured for further identification.

The presence of faecal streptococci in azide or tellurite media may be confirmed by subculture on MacConkey Agar. See below for colonial morphology.

Pasteurella Differentiation

The Oxoid CM7 medium may be used to test the ability of *Pasteurella* bacilli to grow on MacConkey Agar in order to differentiate the various species (e.g. Hoogendijk, 1962). *Pasteurella pestis* and *P. pseudotuberculosis* show slight but visible growth after 24 hours at 37° C—but this may disappear after a few days presumably due to autolysis (Wilson & Miles, 1964). *P. multocida* (*P. septica*) and *P. haemolytica* do not produce visible growth.

Pectinolytic Organisms (Stewart, 1962)

Stewart used Oxoid MacConkey Agar as the basis of a selective-diagnostic medium for pectinolytic organisms, in order to isolate soft-rot *Erwinia* species from specimens containing other Enterobacteriaceae. MacConkey Agar-calcium chloride plates (5.2g CM7 powder, 0.4g $CaCl_2$, 75 ml distilled water) overlaid with a pectate-EDTA layer (0.1% EDTA containing 2% sodium polypectate) are inoculated and incubated for 48 hours at 25° C. Lactose fermenting *Erwinia* produce red colonies in shallow pits formed by pectate liquefaction.

COLONIAL CHARACTERISTICS

After 24 hours at 37° C typical colonies are as follows:

Organism	*Colour*	*Remarks*
Escherichia coli	red	non-mucoid
Aerobacter aerogenes	pink	mucoid
Enterococcus (i.e. faecal streptococcus)	red	minute, round
Staphylococcus	pale pink	opaque
Pseudomonas aeruginosa	green-brown	fluorescent growth

These characteristics are variable and suspected colonies should be subcultured in Peptone Water CM9 or Tryptone Water CM87 for differential tests.

REFERENCES

Hoogendijk, J. L. (1962) *Pasteurella* Strains Isolated from Human Sputum' *Antonie van Leeuwenhoek J. Microbiol. Serol.* **28**(3), 315–320.

Dept. of Health & Social Security (1969) 'The Bacteriological Examination of Water Supplies' 4th ed., H.M.S.O., London.

Stewart, D. J. (1962) 'A Selective-Diagnostic Medium for the Isolation of Pectinolytic Organisms in the *Enterobacteriaceae*' *Nature* **195**(4845), 1023.

Wilson, G. S. and Miles, A. A. (1964) 'Topley and Wilson's Principles of Bacteriology and Immunity' 5th ed., Edward Arnold Ltd., London vol. 2.

Windle Taylor, E. (1958) 'The Examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London.

World Health Organization (1963) 'International Standards for Drinking-Water' 2nd ed., WHO, Geneva.

MacConkey Agar (WITHOUT SALT)

Code CM7b

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**20.0**
Lactose		**10.0**
Bile Salts (Oxoid L55)		**5.0**
Neutral red		**0.075**
Agar No. 3 (Oxoid L13)		**12.0**

pH 7.4 (approx.)

DIRECTIONS

Suspend 47 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring. Dry the surface of the gel before inoculation.

DESCRIPTION

This medium has the same formulation as MacConkey Agar CM7 except that it does not contain added salt and therefore provides a 'low electrolyte medium' on which most *Proteus* species do not spread. For this reason the medium has found particular favour for use in the examination of urine so that overgrowth of other organisms is prevented.

MacConkey Agar No. 2

Code—Powder CM109
Tablets CM110

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**20.0**
Lactose		**10.0**
Bile Salts No. 2		**1.5**
Sodium chloride		**5.0**
Neutral red		**0.05**
Crystal violet		**0.001**
Agar No. 3 (Oxoid L13)		**15.0**

pH 7.2 (approx.)

DIRECTIONS

Powder: Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C. for 15 minutes.

DESCRIPTION

MacConkey Agar No. 2 is a modification of the original MacConkey solid medium and is especially useful for the recognition of enterococci, in the presence of coliforms and non-lactose fermenters from water, sewage, food products, etc.

On this medium faecal streptococci appear as small intensely red colonies with a pale periphery about 1 mm in diameter. These organisms are frequently sought as an index of faecal pollution. Non-lactose fermenters are colourless. Bile tolerant micrococci, such as staphylococci and non-faecal streptococci, are completely inhibited.

McGeachie & Kennedy (1963) employed Oxoid MacConkey Agar No. 2 in a simplifed method for counting the bacteria in urine. Using a bacteriological loop (delivering a known volume) they streaked well mixed uncentrifuged urine directly on to a blood agar and a MacConkey Agar plate—and spread the urine in a 1 cm wide strip across one edge of the plate using 20 strokes. With a second sterile loop they spread a 1 cm wide portion to form a second strip at right angles to the first. This was repeated to give a square pattern of four 1 cm wide strips around the edge of the plate. After incubation, growth was noted as +, ++, +++, or ++++ depending on whether 1, 2, 3 or 4 sides of the square showed colonies. The approximate estimate obtained agreed well with a more complicated pour-plate method and the simplified method was recommended for routine use.

REFERENCE

McGeachie, J. and Kennedy, A. C. (1963) Simplified Quantitative Methods for Bacteriuria and Pyuria' *J. clin. Path.* **16**(1), 32–38.

MacConkey Agar No. 3

Code—Powder CM115
Tablets CM116

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**20.0**
Lactose		**10.0**
Bile Salts No. 3 (Oxoid L56)		**1.5**
Sodium chloride		**5.0**
Neutral red		**0.03**
Crystal violet		**0.001**
Agar No. 3 (Oxoid L13)		**15.0**

pH 7.1 (approx.)

DIRECTIONS

Powder: Suspend 51.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A highly selective modification of MacConkey medium which is suitable for the detection and enumeration of coliform organisms and also for the detection and isolation of *Salmonella* and *Shigella* species occurring in pathological and food specimens. Due to the inclusion of a specially prepared fraction of bile salts in addition to crystal violet, the medium gives improved differentiation between coliforms and non-lactose fermenting organisms whilst Gram-positive cocci are completely inhibited.

This formulation corresponds with that recommended by the American Public Health Association (1946) for the direct plating of water samples for coliform bacilli, the American Public Health Association (1963) for the examination of food samples for food poisoning organisms, and the American Public Health Association (1953) for the isolation of *Salmonella* and *Shigella* species in cheese.

Amongst other examples of the use of Oxoid MacConkey Agar No. 3 are those of: Barnes & Goldberg (1962) for the count of *coli-aerogenes* bacteria in poultry caecal specimens; Medrek and Barnes (1962) for the count of *coli-aerogenes* bacteria in cattle and sheep faeces; Barnes and Shrimpton (1957) for the count of *coli-aerogenes* and non-lactose fermenting organisms in poultry carcases; Thornley (1957) for bacterial counts on irradiated canned minced chicken; Eddy (1960) who used it in conjunction with other media for the recognition of *coli-aerogenes* bacteria during investigations on the genus *Aeromonas*.

TECHNIQUE

After inoculation the plates are usually incubated for 18 to 24 hours at 37° C and for a further 24 hours if non-lactose fermenting organisms are sought and have not appeared. Lower incubation temperatures may sometimes be used for more psychrophilic species. After 18 hours at 37° C, coliforms produce intense violet-red colonies whilst non-lactose fermenters are colourless.

REFERENCES

American Public Health Association (1946) 'Standard Methods for the Examination of Water and Sewage' 9th ed., APHA Inc., New York.

American Public Health Association (1963) 'Diagnostic Procedures and Reagents' 4th ed., APHA Inc., New York.

American Public Health Association (1953) 'Standard Methods for the Examination of Dairy Products' 10th ed., APHA Inc., New York.

Barnes, Ella M. and Goldberg, H. S. (1962) 'The Isolation of Anaerobic Gram-Negative Bacteria from Poultry reared with and without Antibiotic Supplements' *J. appl. Bact.* **25**(1), 94–106.

Barnes, Ella M. and Shrimpton, D. H. (1957) 'Causes of Greening of Uneviscerated Poultry Carcasses during Storage' *J. appl. Bact.* **20**(2), 273–285.

Eddy, B. P. (1960) 'Cephalotrichous, Fermentative Gram-Negative Bacteria: the genus *Aeromonas*' *J. appl. Bact.* **23**(2), 216–249.

Medrek, T. F. and Barnes, Ella M. (1962) 'The Distribution of Group D Streptococci in Cattle and Sheep' *J. appl. Bact.* **25**(2), 159–168.

Thornley, Margaret J. (1957) 'Observations on the Microflora of Minced Chicken Meat Irradiated with 4 MeV Cathode Rays' *J. appl. Bact.* **20**(2), 286–298.

MacConkey Agar No. 3 (ROLL TUBE)

Code CM116a

FORMULA

Peptone (Oxoid L37)	*grams per litre*	20.0
Lactose		10.0
Bile Salts No. 3 (Oxoid L56)		1.5
Sodium chloride		5.0
Neutral red		0.03
Crystal violet		0.001
Agar No. 3 (Oxoid L13)		20.0

pH 7.1 (approx.)

DIRECTIONS

Tablets: Add 1 tablet to 4.0 or 4.5 ml of distilled water in an Astell roll tube and soak for 5 minutes. Loosely insert the rubber seal and sterilize by autoclaving at 121° C. for 15 minutes. On removal from the autoclave, press home the rubber seal and hold at 50° C if required for immediate inoculation. IT IS ESSENTIAL NOT TO REDUCE THE FINAL CONCENTRATION OF BILE SALTS BELOW 0.15% w/v. THEREFORE THE VOLUME OF THE MIXTURE OF MEDIUM PLUS INOCULUM SHOULD NOT EXCEED 5 ml.

The above instructions take the dilution factor into account and allow for the addition of 1 ml or 0.5 ml of fluid inoculum to 4 ml or 4.5 ml respectively of medium.

DESCRIPTION

This medium is recommended for the counting of coliform organisms by the roll tube technique, and is particularly useful for the enumeration of these organisms in shell fish, etc. Oxoid MacConkey Agar No. 3 (Roll Tube) was used by Reynolds & Wood (1956) for the bacteriological examination of shellfish: it was found that use of the simplified MacConkey medium in conjunction with Astell roll tube apparatus constituted a considerable improvement on the original Clegg & Sherwood (1947) method.

REFERENCES

Clegg, L. F. L. and Sherwood, H. P. (1947) 'The Bacteriological Examination of Molluscan Shellfish' *J. Hyg.*, Camb. 45, 504.

Reynolds, N. and Wood, P. C. (1956) 'Improved Techniques for the Bacteriological Examination of Molluscan Shellfish' *J. appl. Bact.* **19**(1), 20–25.

MacConkey Broth

Code—Powder CM5
Tablets CM6

FORMULA

Peptone (Oxoid L37)	*grams per litre*	20.0
Lactose		10.0
Bile Salts (Oxoid L55)		5.0
Sodium chloride		5.0
Neutral red		0.075

pH 7.4 (approx.)

DIRECTIONS

Powder: To prepare single strength broth, add 40 g to 1 litre of distilled water. Mix well and distribute into containers fitted with Durham tubes. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water, insert a Durham's tube and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

For the past fifty years, MacConkey Broth has been the standard medium for the primary isolation of coliform bacteria, and is still officially recommended for this purpose by the Public Health Laboratory Service Water Committee (1961) and the World Health Organization (1963). The Oxoid product conforms to their specification for water testing and also to the formulation specified by the Dept. of Health (1937) for milk grading. Also see page 177.

The advantages of MacConkey Broth in the presumptive coliform test are the low proportion of false positive reactions (PHLS Water Subcommittee, 1953) and the fact that most strains of *Escherichia coli* produce a positive reaction within 24 hours (Windle Taylor, 1958). Disadvantages, due to variability of the peptone and bile salts contained in the original medium, have been overcome by large scale production, pooling of batches and careful quality control—including titrimetric standardization of the bile salts by a method described by Burman (1955).

The neutral red is pre-tested for the absence of toxic substances before inclusion in the Oxoid medium. Childs & Allen (1953) have shown that some samples of neutral red were inhibitory. For those who prefer, this medium is also available with bromo-cresol purple as the indicator—for details of this alternative medium and the presumptive coliform test see MacConkey Broth-Purple (CM5a).

REFERENCES

Burman, N. P. (1955) 'The Standardisation and Selection of Bile Salt and Peptone for Culture Media used in the Bacteriological Examination of Water' *Proc. Soc. Water Treat. Exam.* 4(1), 10–20 and discussion 20–26.
Childs, Eileen and Allen, L. A. (1953) 'Improved Methods for Determining the Most Probable Number of *Bacterium coli* and of *Streptococcus faecalis*' *J. Hyg.*, Camb. **51** (4), 468–477.
Dept. of Health & Social Security (1937) 'Bacteriological Tests for Graded Milk' Memo. 139/Foods, HMSO, London.
Dept. of Health & Social Security (1969) 'The Bacteriological Examination of Water Supplies' 4th impression, HMSO, London.
Public Health Laboratory Service Water Subcommittee (1953) 'The Effect of Anaerobic Spore-Bearing Organisms on the Validity of the Presumptive Coliform Test as used in the Bacteriological Examination of Water' *J. Hyg.*, Camb. **51** (2), 268–277.
Windle Taylor, E. (1958) 'The Examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London.
World Health Organization (1963) 'International Standards for Drinking-Water' 2nd ed., WHO, Geneva.

MacConkey Broth (PURPLE)

Code—Powder CM5a
Tablets CM6a

FORMULA

Peptone (Oxoid L37)	*grams per litre*	20.0
Lactose		10.0
Bile Salts (Oxoid L55)		5.0
Sodium chloride		5.0
Bromo-cresol purple		0.01

pH 7.4 (approx.)

DIRECTIONS

Powder: To prepare single strength broth add 40 g to 1 litre of distilled water. Distribute into containers fitted with Durham's tubes. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water. Insert a Durham tube and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

MacConkey Broth has long been used as a presumptive medium for the detection of the *coli-aerogenes* organisms. In the original medium, litmus was employed as the indicator of acid production but, in later publications, MacConkey suggested neutral red as a more satisfactory alternative. More recently, Childs & Allen (1953) showed that some samples of neutral red exerted an inhibitory effect on the growth of *Escherichia coli* in this medium.

Bromo-cresol purple is less inhibitory, and the colour change from purple to yellow provides a more sensitive and definite indication of acid formation; therefore this indicator is used in Oxoid MacConkey Broth-Purple, which corresponds to the alternative formulations recommended in 'The Bacteriological Examination of Water Supplies' (1969) and 'International Standards for Drinking Water' (1963).

TECHNIQUE

The presumptive coliform examination consists of the inoculation of measured volumes of water into tubes of MacConkey Broth-Purple which are incubated at 37° C for 48 hours. Choice of volumes for inoculation will depend on the bacteriological grade of the water being tested; for 'medium' waters the Public Health Laboratory Service Water Committee (1961) recommend one 50 ml, five 10 ml and five 1 ml quantities of water—50 ml and 10 ml amounts being added to their own volume of double-strength MacConkey Broth while the 1 ml amounts are each added to 5 ml of single-strength MacConkey Broth. Acid formation is indicated by a yellow colouration of the broth, and gas formation is indicated by an amount of gas at least sufficient to fill the concavity at the top of the Durham tube. From the number of tubes showing the presence of acid and gas, the most probable number of (presumed) coliform bacteria present in 100 ml of the original water may be estimated by reference to probability tables; these tables based on McCrady's computations, are included in Report No. 71: 'The Bacteriological Examination of Water Supplies' (1969) and in many other publications dealing with this subject. For the differential coliform test, each MacConkey tube showing acid and gas is then subcultured into a fresh tube of MacConkey Broth and incubated at 44° C. Formation of gas within 48 hours is practically specific for *Escherichia coli* (*Bacterium coli* type 1) and indicative of faecal pollution of the original water sample.

MacConkey Broth-Purple is also suitable for the bacteriological examination of milk, as described by Davis (1959). This method, which is basically similar to that used for the examination of water, consisting in the inoculation of suitable dilutions of the milk into tubes of this medium followed by incubation and inspection, was originally recommended by the Dept. of Health & Social Security London (1937).

REFERENCES

Childs, Eileen and Allen, L. A. (1953) 'Improved Methods for Determining the Most Probable Number of *Bacterium coli* and of *Streptococcus faecalis*' *J. Hyg.*, Camb. **51** (4), 468–477.
Davis, J. G. (1959) 'Milk Testing' 2nd ed., Dairy Industries Ltd., London.
Dept. of Health & Social Security (1937) 'Bacteriological Tests for Graded Milk' Memo. 139/Foods, HMSO, London.
Dept. of Health & Social Security (1969) 'The Bacteriological Examination of Water Supplies' 4th ed, HMSO, London.
World Health Organization (1963) 'International Standards for Drinking-Water' 2nd ed., WHO, Geneva.

MacConkey Membrane Broth Tablets

Code MM6a

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10.0**
Lactose		**30.0**
Bile Salts No. 3 (Oxoid L56)		**4.0**
Sodium chloride		**5.0**
Bromo-cresol purple		**0.12**

pH 7.4 (approx.)

For further details of membrane filtration techniques, see the separate Oxoid booklet entitled 'Membrane Filtration'.

Malt Extract

Code L39

Malt Extract is frequently employed as an ingredient of mycological culture media. It is prepared by extracting the solubles from sprouted grain followed by low temperature evaporation to dryness, thus conserving the nitrogenous and carbohydrate constituents. Malt Extract exhibits no diastatic activity and is recommended for the preparation of wort media.

An average analysis of Malt Extract is as follows:

Maltose	52.2%
Dextrose	19.1%
Sucrose	1.8%
Dextrin	15.0%
Other carbohydrates	3.8%
Protein	4.6%
Ash	1.5%
Moisture	2.0%

Malt Extract Agar

Code—Powder CM59
Tablets CM60

FORMULA

Malt Extract (Oxoid L39)	*grams per litre*	**30**
Mycological Peptone (Oxoid L40)		**5**
Agar No. 1 (Oxoid L11)		**15**

pH 5.4 (approx.)

DIRECTIONS

Powder: Suspend 50 grams in 1 litre of distilled water and boil to dissolve. Sterilize by autoclaving at 115° C. for 10 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and autoclave at 115° C for 10 minutes.

If it is desired to adjust the reaction to pH 3.5, cool to 55° C and add 2 ml of 10% Lactic Acid SR21 to every 100 ml of Malt Extract Agar. Once acidified with lactic acid, the medium should not be re-heated.

DESCRIPTION

This medium, similar to the one described by Galloway & Burgess (1952), is recommended for the detection, isolation and enumeration of yeasts and moulds. For mycological counts it may be desirable to prepare the more acid medium in order to suppress bacterial growth.

Also see Wort Agar, page 253.

REFERENCE

Galloway, L. D. and Burgess, R. (1952) 'Applied Mycology and Bacteriology' 3rd ed., Leonard Hill, London, pp. 54 and 57.

Malt Extract Broth

Code—Powder CM57
Tablets CM58

FORMULA

Malt Extract (Oxoid L39)	*grams per litre*	**17**
Mycological Peptone (Oxoid L40)		**3**

pH 5.4 (approx.)

DIRECTIONS

Powder: Add 20 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 115° C for 10 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 115° C for 10 minutes.

This liquid medium is recommended for the cultivation of moulds and yeasts, during tests for sterility, etc.

Maltose 10 %

Code SR17

Filter sterilised solution, available in 5 ml ampoules, in boxes of ten.

Mannitol 10 %

Code SR14

Filter sterilized solution, available in 5 ml ampoules, in boxes of ten.

Mannitol Salt Agar

Code CM85

FORMULA

'Lab-Lemco' Powder (Oxoid L29) *grams per litre*	**1.0**
Peptone (Oxoid L37)	**10.0**
Mannitol	**10.0**
Sodium chloride	**75.0**
Phenol red	**0.025**
Agar No. 3 (Oxoid L13)	**15.0**

pH 7.5 (approx.)

DIRECTIONS

Suspend 111 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A selective medium prepared according to the recommendations of Chapman (1945) for the isolation of presumptive pathogenic staphylococci. Most other bacteria are inhibited by the high salt concentration with the exception of some halophilic marine organisms. Presumptive coagulase-positive staphylococci produce colonies surrounded by bright yellow zones whilst non-pathogenic staphylococci produce colonies with reddish purple zones.

Mannitol Salt Agar is recommended for the detection and enumeration of coagulase-positive staphylococci in milk (Davis, 1959), in food (American Public Health Association, 1966) and other specimens (e.g. Silverton and Anderson, 1961).

Amongst those who have employed Oxoid Mannitol Salt Agar for the examination of meat or fish are: Barnes & Shrimpton (1957), Thornley (1957), Bain *et al.* (1958), Spencer (1961) and Eddy & Ingram (1962).

TECHNIQUE

Heavily inoculate the Mannitol Salt Agar plate and incubate for 36 hours at 37° C for 3 days at 32° C—the latter is recommended by the APHA (1958).

Presumptive coagulase-positive staphylococci produce colonies with bright yellow zones whilst coagulase-negative staphylococci are surrounded by a red or purple zone. Pick off suspect colonies and subculture in a medium not containing an excess of salt (e.g. Nutrient Broth No. 2 CM67) to avoid interference with coagulase or other diagnostic tests.

REFERENCES

American Public Health Association (1966) 'Recommended Methods for the Microbiological Examination of Foods' 2nd ed. APHA Inc., New York.

Bain, Nora, Hodgkiss, W.; Shewan, J. M. (1958) 'The Bacteriology of Brines used in Smoke Curing of Fish' DSIR, Proc. 2nd Internat. Symp. Food Microbiol., 1957, HMSO, London, pp. 103–116.

Barnes, Ella M. and Shrimpton, D. H. (1957) 'Causes of Greening of Uneviscerated Poultry Carcasses during Storage' *J. appl. Bact.* **20**(2), 273–285.

Chapman, G. H. (1945) 'The Significance of Sodium Chloride in Studies of Staphylococci' *J. Bact.* **50**, 201–203.

Davis, J. G. (1959) 'Milk Testing' 2nd ed., Dairy Industries Ltd., London.

Eddy, B. P. and Ingram, M. (1962) 'The Occurrence and Growth of Staphylococci on Packed Bacon, with special reference to *Staphylococcus aureus J. appl. Bact.* **25**(2), 237–247.

Silverton, R. E. and Anderson, M. J. (1961) 'Handbook of Medical Laboratory Formulae Butterworths, London.

Spencer, R. (1961) 'The Bacteriology of Distant Water Cod Landed at Hull' *J. appl. Bact.* **24**(1), 4–11.

Thornley, Margaret J. (1957) 'Observations on the Microflora of Minced Chicken Meat Irradiated with 4MeV Cathode Rays' *J. appl. Bact.* **20**, 286–298.

Mannitol Selenite Broth

Code CM39a

This medium has been withdrawn as the Sodium biselenite in the medium may constitute a health hazard. A leaflet explaining Oxoid's reasons for this decision is available on request.

See **MANNITOL SELENITE BROTH BASE**
CM399/Sodium Biselenite L121

Mannitol Selenite Broth Base

(*Selenite Broth Base,* see page 241)

Code CM399—Sodium Biselenite Code L121

FORMULA

Bacteriological Peptone (Oxoid L34) *grams per litre*	**5.0**
Mannitol	**4.0**
Sodium phosphate	**10.0**

DIRECTIONS

Add 19 grams to 1 litre of distilled water to which 4 grams of sodium biselenite (Oxoid L121) has been added.

Warm to dissolve, mix well and fill out into containers to a depth of 5 cm. Sterilize in a boiling water bath, or in free flowing steam, for 10 minutes. DO NOT AUTOCLAVE.

To minimise any possible risk of teratogenicity to laboratory workers, the sodium biselenite must be added to this medium separately.

DESCRIPTION

This medium is similar to the modification of Leifson enrichment medium (1936) described by Hobbs & Allison (1945) for the isolation of *Salmonella typhosa* and *Salmonella paratyphi B.*

Hobbs & Allison (1945) compared two sets of selenite media, one containing lactose and the other mannitol. Of 38 positive stools *S. typhosa* was subcultured from both media in 32 instances, from the mannitol selenite alone in 5 instances and from the lactose selenite alone once. Comparisons showed that the mannitol selenite broth was superior to three other liquid media in its selective value for *S. typhosa*, and that it was as good as tetrathionate for the isolation of *S. paratyphi B.*

Arnold (1956) slightly modified the Hobbs and Allison medium by the use of sodium selenite instead of sodium biselenite and by bringing the pH down to 7.0. The Arnold medium was satisfactory for the examination of sewage swabs for *Salmonella* organisms.

TECHNIQUE

This medium is used in the same manner as Selenite Broth Base CM395 (see page 213) in conjunction with Bismuth Sulphite Agar CM201 and Brilliant Green Agar CM263, Desoxycholate Citrate Agar CM35, Desoxycholate Citrate Agar (Hynes) CM227, or S.S. Agar CM99.

REFERENCES

Arnold, J. B. (1955–56) 'A Modified Technique for the Examination of Sewage Swabs for *Salmonella* Organisms' *J. Med. Lab. Technol.* **13,** 540–542.

Hobbs, Betty C. and Allison, V. D. (1945) 'Studies on the Isolation of *Bact. typhosum* and *Bact. paratyphosum B*' *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv.* **4,** 12–19.

Leifson, E. (1936) 'A New Selenite Enrichment Media for the Isolation of Typhoid and Paratyphiod (*Salmonella*) Bacilli' *Am. J. Hyg.* **24**(2), 423–432.

Membrane Enriched Teepol Broth

Code MM369

FORMULA

Bacteriological Peptone (Oxoid L34)	*grams per litre*	**40.0**
Yeast Extract (Oxoid L21)		**6.0**
Lactose		**30.0**
Phenol Red		**0.2**

pH 7.4 (approx.)

DIRECTIONS

Dissolve 76.2 grams in 1 litre of distilled water containing 4 ml of Teepol 610 (BDH Ltd.). Distribute into storage containers, e.g. 100 ml screw-cap bottles. Sterilize by steaming for 30 minutes on three consecutive days or autoclaving at 121° C for 15 minutes.

DESCRIPTION

In formulating Membrane Enriched Teepol Broth, Burman (1967a) substituted Teepol in place of bile salts in the membrane filtration test medium used to detect coliform organisms in water. The use of Teepol in place of bile salts had been previously recommended by Jameson and Emberley (1956) and its value was confirmed by other workers (Jebb 1959, Windle-Taylor 1959–60, 1961–62). It is essential to use one standard grade of Teepol and Teepol 610 (BDH Ltd.) has been recommended.

Burman (1967b) showed that resuscitation media are not required with Membrane Enriched Teepol Broth if a preliminary incubation is carried out at a lower temperature. Thus non-chlorinated organisms benefit from 4 hours incubation at 30° C, but chlorinated organisms require 6 hours incubation at 25° C.

Although several modifications of the medium were tried Burman showed that Membrane Enriched Teepol Broth, after the resuscitation procedure, gave higher counts of Esch. coli and coliform organisms than the classical M.P.N. method in fluid media.

The Medium and method are fully described in The Bacteriological Examination of Water Supplies Report 71, 1969.

TECHNIQUE

The coliform and Esch. coli count are made on separate volumes of water. The volumes should be chosen so that the number of colonies to be counted on the membrane lies between 10 and 100. If the water is suspected to contain less than 100 coliform organisms per 100 ml, then a 100 ml sample should be filtered.

The water samples are filtered through a sterile Oxoid membrane filter (Report 71, 1969) and the membrane filter is placed face upwards on an absorbent pad previously saturated with Membrane Enriched Teepol Broth. The pad and membrane filter should be incubated in a vapour-tight container to prevent evaporation.

Membranes to be incubated at 44° C should be placed in watertight heavy containers and immersed in a closely controlled water-bath.

Burman recommends the following incubation periods and temperatures:

UNCHLORINATED WATERS

Coliform organisms—	4 hours at 30° C followed by 14 hours at 35° C.
Esch. coli	4 hours at 30° C followed by 14 hours at 44° C.

If rapid results are required, the membrane may be examined after a total incubation time of 12 hours. If no colonies are present, a nil count can be assumed. If small colonies of indeterminate colour are present, then the membranes must be returned to the incubator for the full period.

PRESUMPTIVE COLIFORM ORGANISMS

After incubation, yellow colonies from membranes incubated at 35° C or 37° C should be sub-cultured to Lactose Peptone Water to confirm that they will produce gas at 37° C after 48 hours incubation.

PRESUMPTIVE ESCH. COLI.

Yellow colonies from membranes incubated at 44° C should be sub-cultured to Lactose Peptone Water, or Lactose Ricinoleate Broth and to Tryptone Water to confirm gas and indole production at 44° C after 24 hours incubation.

REFERENCES

Burman, N. P. (1967a) *Proc. Soc. Wat. Treat. Exam.* **16**, 40.

Burman, N. P. (1967b) 'Rec. Adv. in Bacteriological Examination of Water. Progress in Microbiological Techniques' edited by C. H. Collins. London. Butterworth, p. 185.
Jameson, J. E. and Emberley, N. W. (1956) 'A substitute for bile salts in culture media' *J. gen. Microbiol.* **15**, 198–204.
Jebb, W. H. H. (1959) 'A comparison of media for the detection of coliform organisms in water' *J. Hyg.*, Camb. **57**, 184–192.
Report 71 (1969) 'The Bacteriological Examination of Water Supplies' 4th ed., London, HMSO.
Windle Taylor, E. (1959–60) 'Glutamic acid media' 39th Ann. Rep. Dir. Water Exam. Met. Water Board, London, pp. 27–30.
Windle Taylor, E. (1961–62) 'Glutamic acid medium 40th Ann. Rep. Dir. Water Exam. Met. Water Board, London, pp. 18–22.

Membrane Filtration

We supply an extensive range of microporous cellulose acetate membranes, special culture media, incubating pads and tins for use primarily in microbiological membrane filter techniques. The special culture media known as 'membrane media' are described briefly in alphabetical order in the text of this Manual:

Membrane Enriched Teepol Broth
MacConkey Membrane Broth
Resuscitation Membrane Broth

For more details of membrane filtration media, apparatus and techniques—see the separate Oxoid booklet.

Milk Agar

Code—Powder CM21
Tablets CM22

FORMULA

Yeast Extract (Oxoid L20)	*grams per litre*	**3**
Peptone (Oxoid L37)		**5**
Milk solids (equivalent to 10 ml fresh milk)		**1**
Agar No. 3 (Oxoid L13)		**15**

pH 7.2 (approx.)

DIRECTIONS

Powder: Suspend 24 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 5 ml of distilled water, soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Oxoid Milk Agar is made to a formula corresponding to the official medium described in Dept. of Health & Social Security Memo. 139/Foods (1937). It is recommended for performing the plate count test on milks, rinse waters, milk products and ice cream, etc. This medium has been thoroughly tested against the official medium and has been shown to give identical results.

TECHNIQUE

The sample bottle is inverted 25 times, and, after flaming the mouth, some of the milk sample is discarded—while the remainder is re-shaken thoroughly and used for the preparation of decimal dilutions in ¼ strength Ringer solution.

For milk, dilutions of 1/10, 1/100 and 1/1,000 are prepared and 1 ml of each pipetted aseptically into separate Petri dishes. 10 ml of molten Milk Agar, cooled to 45° C, is then added to each dish and the contents mixed by a combination of rapid to-and-fro shaking and circular movements lasting 5–10 seconds. The recommended procedure is five to-and-fro movements followed by five circular movements in a clockwise direction, succeeded by five to-and-fro movements at right angles to the first set followed by five anti-clockwise circular movements.

No more than fifteen minutes should elapse between preparation of the dilutions and pouring the plates.

The plates are allowed to stand on the bench for about an hour and then transferred to the incubator, where they are incubated in an inverted position for 2 days at 37° C or 3 days at 30° C (Davis, 1959). Thomas and Jenkins (1940) and Wilson *et al.* (1935) have shown that appreciably higher counts may be obtained after incubation at 22° C and 30° C than at 37° C.

After incubation the colonies are counted, within four hours, and the result expressed as plate count per ml.

REFERENCES

Davis, J. G. (1959) 'Milk Testing' 2nd ed., Dairy Industries Ltd., London, pp. 175–187.
Dept. of Health & Social Security (1937) 'Bacteriological Tests for Graded Milk' Memo. 139/Foods.
Thomas, S. B. and Jenkins, E. 'Colony counts on Milk Agar incubated at 25°, 30° and 37°C', (1940) *Proc. Soc. App. Agric.*, 38–40.
Wilson, G. S. (1935) 'Bacteriological Grading of Milk' HMSO, London.

Milk Agar (ROLL TUBE)

Code—Powder CM21a
Tablets CM22a

FORMULA

Yeast Extract (Oxoid L20)	*grams per litre*	**3**
Peptone (Oxoid L37)		**5**
Milk solids (equivalent to 10 ml fresh milk)		**1**
Agar No. 3 (Oxoid L13)		**20**

pH 7.2 (approx.)

DIRECTIONS

Powder: Suspend 29 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Distribute 4.0 or 4.5 ml amounts into Astell tubes and loosely insert the seals. Sterilize by autoclaving at 121° C for 15 minutes. Immediately on removal from the autoclave, press home the seals.
Tablets: Add 1 tablet to 4.0–4.5 ml of distilled water in an Astell bottle and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes. Immediately on removal from the autoclave, press home the seal.

DESCRIPTION

Milk Agar (Roll Tube) is made to a formula corresponding to the official medium described in Dept. of Health & Social Security Memo. 139/Foods

(1937). It is recommended for making colony counts on milk, rinse waters, milk products and ice cream, etc., using the roll-tube technique.

TECHNIQUE

The roll-tube method possesses many advantages over plating techniques for the enumeration of viable organisms. Petri dishes are replaced by special tubes in which the inoculum is mixed with melted agar medium. The tubes are spun until the agar sets in an even film on the wall of the tubes, and after incubation the colonies are counted as with a normal plate count. The roll tube method effects considerable economies in time, glassware, media, labour costs, and space in sterilizers and incubators.

Whilst still liquid, the bottles of sterile medium are transferred to a water bath at 43–45° C in order to cool the medium ready for inoculation. 0.5 or 1.0 ml of diluted milk is introduced into each bottle, which is immediately sealed and placed on the spinning machine. The spun bottle is removed after solidification of the medium, incubated seal downwards for 2 days at 37° C (see note on time and temperature of incubation under Milk Agar), and the number of colonies counted.

A range of roll-tubes and apparatus is supplied by the Astell Laboratory Service Co. Ltd., from whom further details may be obtained.

REFERENCE

Dept. of Health & Social Security (1937) 'Bacteriological Tests for Graded Milk' Memo. 139/Foods, HMSO, London.

Minerals Modified Glutamate Medium

Code CM289

FORMULA

	(double strength)
Sodium glutamate . . . . *grams per litre*	12.7
Lactose	20.0
Sodium formate	0.5
L-cystine	0.04
L(−)aspartic acid	0.048
L(+)arginine	0.04
Thiamine	0.002
Nicotinic acid	0.002
Pantothenic acid	0.002
Magnesium sulphate ($MgSO_4$ $7H_2O$)	0.200
Ferric ammonium citrate	0.020
Calcium chloride ($CaCl_2$ $2H_2O$)	0.020
Di-potassium hydrogen phosphate	1.80
Brom-cresol purple	0.020

pH 6.7 (approx.)

DIRECTIONS

Double Strength—dissolve 35.4 grams in 1 litre of distilled water containing 5 grams ammonium chloride.

Single Strength—dissolve 17.7 grams in 1 litre of distilled water containing 2.5 grams ammonium chloride.

Sterilize by autoclaving for 10 minutes at 116° C, alternatively heat to 100° C for 30 minutes on three successive days.

DESCRIPTION

A chemically defined medium based on glutamic acid was first advocated by Folpmers (1948) for the enumeration of the coliform group of bacteria in water. The Public Health Laboratory Service (1958) carried out a trial and concluded that glutamic acid media containing glucose gave too many false positives in 48 hours.

Gray (1959) modified a lactose containing glutamate medium and later (1964) published an improved formate lactose glutamate medium.

This latter medium was incorporated in a large trial carried out by the P.H.L.S. (1968) in which three glutamate media were compared with Teepol Broth (Jameson & Emberly, 1956) and MacConkey Broth. The trial showed that Gray's improved formate lactose glutamate medium was superior to the other media in the trial.

There was some criticism of the mineral content of the medium in the report and it was considered that the medium could be improved by modifying the amounts of minerals.

A co-operative investigation carried out between the Metropolitan Water Board Laboratories and Oxoid Laboratories resulted in a Minerals Modified Glutamate Medium.

The Oxoid Minerals Modified Glutamate Medium was used in the P.H.L.S. (1969) trial and the results with the Oxoid medium confirmed the superior performance reported previously (P.H.L.S., 1968).

The superior performance of Minerals Modified Glutamate Medium over MacConkey Broth is due mainly to improved detection of Escherichia coli. The table (adapted from P.H.L.S., 1969) illustrates the results obtained in the trial.

Comparison of Minerals Modified Glutamate Medium and MacConkey Broth by numbers of positive tubes.

	Number of tubes yielding								
	False positive reactions			*Coliform organisms*			*Esch. Coli*		
	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.	18 hr.	24 hr.	48 hr.
Unchlorinated samples (no. of tubes examined 2079)									
MacConkey Broth	17	37	100	625	806	1060	467	528	582
Minerals Modified Glutamate Medium	2	20	97	557	858	1175	503	707	764
Chlorinated samples (no. of tubes examined 715)									
MacConkey Broth	4	19	49	125	216	315	77	121	128
Minerals Modified Glutamate Medium	0	1	37	59	223	395	39	144	203

The table shows that for chlorinated water, incubation for 24 hours is required for glutamate media to demonstrate their superiority. The pH of the final medium is critical for optimum performance and the sterilized broth should be checked to confirm that it is at pH 6.7 before use. Tests carried out in various laboratories showed that differences in heating procedures caused differences in final pH value. If necessary the heating procedure should be adjusted so that the final pH, after sterilization is 6.7.

The medium and method are fully described in The Bacteriological Examination of Water (Report 71, 1969).

TECHNIQUE

The technique known as the Multiple Tube Method, Dilution Method or the Most Probable Number (MPN) method is used with Minerals Modified Glutamate Medium.

With waters expected to be of good quality, one 50 ml volume and five 10 ml volumes of water should be inoculated into the medium. With waters of more doubtful quality, five 1 ml volumes should be used in addition to the 50 ml and 10 ml volumes. Dilutions of the 1 ml volumes may be required for polluted water and the 50 ml volume may be omitted.

The larger volumes of water (10 ml and 50 ml) are added to equal volumes of double-strength medium. Whereas the 1 ml volumes (or dilutions thereof) are added to 5 ml of single-strength medium.

The tubes are incubated at 37° C and examined after 18–24 hours. All those tubes showing acid (yellow colour in medium) and gas in the inverted inner (Durham) tube should be regarded as 'presumptive positive' tubes, including those in which gas appears after tapping the tube. The tube may only have a bubble of gas after tapping. The remaining tubes should be re-incubated and examined after another 24 hours. Any further tubes becoming 'positive' should be treated as 'presumptive positives'.

Each 'presumptive positive' tube should be sub-cultured to a tube of either Lactose Ricinoleate Broth (CM371) or Brilliant Green Bile (2%) Broth (CM31) and incubated for 24 hours at 44° C.

At the same time a tube of 1% Tryptone Water (CM87) should be inoculated for the production of indole after 24 hours at 44° C.

The production of gas from lactose at 44° C and the production of indole at 44° C are accepted in the United Kingdom as evidence of *Escherichia coli*.

Samples of chlorinated water giving 'presumptive positive' tubes must be tested to exclude false positive results due to aerobic or anaerobic gas producing spore bearing organisms. Sub-cultures are made into Lactose Ricinoleate Broth or Brilliant Green Bile (2%) Broth and incubated at 37° C. for 48 hours. Production of gas in these media within 48 hours can be taken as sufficient confirmation that coliform organisms are present. If the tubes are sub-cultured to MacConkey Agar (CM7) at the same time, the colonial morphology of the organisms can be seen and pure cultures can easily be obtained for further differential tests.

The Most Probable Number of organisms can be calculated from the tables in Appendix C of Report 71 (1969).

Minerals Modified Glutamate Medium was developed for the enumeration of coliform organisms in water. Similar results may not obtain in milk or other food-stuffs where the addition of these nutrient compounds may influence the behaviour of the medium (P.H.L.S., 1969).

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P.H.L.S. Standing Committee on Bacteriological Examination of Water Supplies (1968) 'Comparison of MacConkey Broth, Teepol Broth and Glutamic Acid Media for the enumeration of coliform organisms in water' *J. Hyg.*, Camb. **66**, 67–82.

Report 71 (1969) 'The Bacteriological Examination of Water Supplies' 4th ed., London, HMSO.

Mitis Salivarius Agar Base

Code CM157

FORMULA

	grams per litre
Peptone P (Oxoid L49)	**10.0**
Tryptone (Oxoid L42)	**10.0**
Dextrose	**1.0**
Sucrose	**50.0**
Dipotassium phosphate	**4.0**
Trypan blue	**0.075**
Crystal violet	**0.0008**
Agar No. 1 (Oxoid L11)	**12.0**

pH 7.0 (approx.)

DIRECTIONS

Suspend 87 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C and add 1 ml of 1% Potassium Tellurite (prepared by adding one 2 ml ampoule of 3.5% Potassium Tellurite SR30 to 5 ml of distilled water). Mix well before pouring. DO NOT REHEAT.

DESCRIPTION

Oxoid Mitis Salivarious Agar is similar to the medium described by Chapman (1944, 1947) for the isolation of streptococci from specimens containing a high proportion of other micro-organisms. It is an inhibitory and differential medium, being actually lethal to most coliform bacilli and providing good colonial differentiation between *Streptococcus mitis* and *Streptococcus salivarius*. Chapman found that 97% of all cultures from faeces on the medium consisted of pure cultures of streptococci and that most of the remaining 3% were pure or mixed cultures of staphylococci. He reported that spreading organisms had not been observed on this medium.

Williams *et al.* (1956) concluded that *Str. salivarius* and other *viridans*-type streptococci could be used as a valid indication of respiratory pollution of air. Reid *et al.* (1956) tested the correlation between *Str. salivarius* counts and bacterial air pollution.

TECHNIQUE

Incubation for exactly 24 hours at 37° C is recommended, but occasionally with the *mitis* type, it is desirable to incubate for 48 hours.

COLONIAL APPEARANCE

After 24 hours' incubation, typical colonies may be distinguished as follows:

Streptococcus mitis (and beta-haemolytic streptococci)—small blue colonies about 0.2 mm diameter.

Streptococcus salivarius—pale blue, opaque colonies 2–5 mm diameter. Usually smooth, raised, glistening colonies with a 'gum-drop' appearance.

Enterococci—dark blue or black, shiny, slightly raised colonies 1–2 mm diameter.

Coliforms (which occasionally grow)—brown colonies.

REFERENCES

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M.R.S. Agar

(de Man, Rogosa, Sharpe)
Code CM361

FORMULA

As for M.R.S. Broth with the addition of 10 grams Agar No. 1 (Oxoid L11).

DIRECTIONS

Suspend 62 grams in 1 litre of distilled water. Boil to dissolve the medium completely. Dispense into tubes, bottles or flasks and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

The M.R.S. formulation was developed by de Man, Rogosa and Sharpe (1960) to replace a variable product (tomato juice) and at the same time to provide a medium which would support good growth of lactobacilli in general, even those strains which showed poor growth in existing media.

M.R.S. medium is superior to the tomato juice medium of Briggs (1953) and the meat extract tomato juice medium of de Man (1956). It gives more

profuse growth of all trains of lactobacilli, especially the difficult and slow growing strains of *L. brevis* and *L. fermenti.*

In their selection of peptone for M.R.S. medium, de Man *et al.* (1960) found Oxoid peptone to be superior to the others tested. They were able to show virtually the same growth levels when six batches of Oxoid peptone were compared with each other. Since it was the aim of the authors to use standardised ingredients for the medium, Oxoid peptone, Oxoid meat extract (Lab-Lemco) and Oxoid yeast extract were incorporated into their final formulation.

A further step forward in the standardization of M.R.S. media was the production of Oxoid M.R.S. Broth and Agar in dehydrated form.

The nutritional requirements for lactobacilli are complex and optimal growth is only obtained in media such as M.R.S. which contain a fermentable substrate and adequate growth factors.

The lactobacilli are micro-aerophilic and generally require layer plates for aerobic cultivation on solid media. Submerged or surface colonies may be compact or feathery, and are small, opaque and white.

TECHNIQUE

Products to be examined for lactobacilli content are macerated or diluted in a diluent such as quarter-strength Ringer solution, and further dilutions are made in M.R.S. broth.

1 ml volumes of the diluted samples are added to sterile petri dishes, and molten M.R.S. agar (45° C) is poured into the dish and mixed thoroughly. When the medium has set, another layer of uninoculated M.R.S. agar is poured over the surface to produce a layer-plate.

Plates are incubated at 37° C for 3 days or at 30° C for 5 days. It is mportant that adequate moisture vapour is present in the atmosphere above the agar because drying of the plates during incubation will concentrate the selective factors on the surface and make the medium inhibitory. The presence of carbon dioxide stimulates growth and plates should be incubated in an atmosphere of 5% CO_2. M.R.S. medium is selective for lactobacilli but some growth of leuconostocs and pediococci may occur.

Select isolated colonies on the agar medium and stain a smear from each to identify the presumptive *Lactobacillus* colonies; pick these off into M.R.S. broth. An advantage of this broth is that any other micro-organisms, originally lying dormant in the selective agar, are not given the opportunity to multiply, as may occur in a non-selective broth. Incubate the broths at temperatures and times similar to those used for the M.R.S. agar; they can then be examined microscopically and further sub-cultured to M.R.S. agar for subsequent confirmation and identification of species.

M.R.S. broth may be used for further tests in the identification of lactobacilli, such as temperature dependence, growth in 4% NaCl, growth in 0.4% Teepol, etc., as recommended by Sharpe, Fryer and Smith (1966).

REFERENCES

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M.R.S. Broth

(de Man, Rogosa, Sharpe)

Code CM359

FORMULA

Bacteriological Peptone (Oxoid L34)	*grams per litre*	**10**
'Lab-Lemco' Powder (Oxoid L29)		**8**
Yeast Extract (Oxoid L21)		**4**
Dextrose		**20**
'Tween' 80		**1 ml**
di-Potassium hydrogen phosphate		**2**
Sodium acetate $3H_2O$		**5**
tri-Ammonium citrate		**2**
Magnesium sulphate $7H_2O$		**0.2**
Manganese sulphate $4H_2O$		**0.05**

pH 6.2 (approx.)

DIRECTIONS

Add 52 grams to 1 litre of distilled water at 60° C. Mix until completely dissolved. Dispense into final containers and sterilize by autoclaving at 121° C for 15 minutes.

MRVP Medium

Code CM43

FORMULA

Peptone P (Oxoid L49)	*grams per litre*	**5**
Dextrose		**5**
Phosphate buffer		**5**

pH 7.5 (approx.)

DIRECTIONS

Add 15 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This glucose-phosphate medium is recommended for the Methyl-red and Voges-Proskauer tests, for the differentiation of the *coli-aerogenes* group, described in Dept. of Health & Social Security Report No. 71 (1969) 'The Bacteriological Examination of Water Supplies'.

Smith (1895) noted the low acid production of *Aerobacter aerogenes* cultures as compared with those of *Escherichia coli.* Clark & Lubs (1915) employed methyl-red as a hydrogen-ion concentration indicator in order to differentiate dextrose phosphate peptone water cultures of members of the coli-typhoid group. This test, now known as the Methyl-red test, distinguishes between organisms which produce and maintain a high acidity, and those producing an initially lower acidity which reverts towards neutrality. The former type of organism, such as *Escherichia coli,* produces a red coloration and is referred to as Methyl-red positive: the latter, such as *Aerobacter aerogenes,* produces a yellowish coloration and is termed methyl-red negative.

Voges & Proskauer (1898) described a red fluorescent coloration which appeared after the addition of potassium hydroxide to cultures of certain organisms in dextrose medium. The coloration was shown (Harden & Walpole, 1906; Harden and Norris, 1911) to be due to the oxidation of the acetylmethyl-carbinol producing diacetyl, which reacts with the peptone of the medium to give a red colour. Durham (1901) noted that *Aerobacter aerogenes* gave a positive reaction but that *Escherichia coli* produced no coloration, and it later became clear that there was a negative correlation between the Methyl-red and Voges-Proskauer tests (Levine, 1916a, b) for lactose fermenting coliform organisms.

TECHNIQUE

Inoculate a 10 ml tube of MRVP Medium with two loopfuls of a pure, 4–6 hours old, Peptone Water (CM9) culture of the organism under test. Incubate for 3 days at 30° C and divide the culture into two equal portions:

Methyl-red Test—to one portion add 5 drops of 0.04% methyl-red solution. A magenta red colour is considered a positive result; a yellow colour negative; pink or pale red are equivocal.

Voges-Proskauer Test—to the other portion add a trace of creatinine and 5 ml of 40% potassium hydroxide. Shake the tube well. A pink colour is positive; no colour is negative.

REFERENCES

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Smith, T. (1895) *Amer. J. Med. Sci.* **110**, 283.
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Mueller Hinton Agar

Code CM337

FORMULA

Beef, infusion from	*grams per litre*	**300.0**
Casein Hydrolysate (Oxoid L41)		**17.5**
Starch		**1.5**
Agar No. 1 (Oxoid L11)		**10.0**

pH 7.4 (approx.)

DIRECTIONS

Suspend 35 grams in 1 litre of distilled water. Bring to the boil to dissolve the medium completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Mueller Hinton Agar was designeed to be a reproducible cultur medium for the isolation of pathogenic *Neisseria* species (Mueller & Hinton, 1941). The inclusion of starch ensures that toxic factors found during growth will be absorbed and its presence is often essential to establish growth from very small inocula.

Mueller Hinton Agar is now widely used for Antibiotic Sensitivity Testing (including sulphonamides). The inclusion of specially selected agar in the Oxoid Mueller Hinton Agar ensures that large, clear zones of inhibition will be evident when sensitive organisms meet active antibiotics and sulphonamides, and that the zone sizes correspond with those of other standard media. For sulphonamide testing, however, it is essential that broth cultures of organisms are diluted at least 100-fold. Sulphonamides may be antagonised by growth factors in the broth cultures and they are susceptible to high inoculation effects, when the organisms may carry sufficient sulphonamide antagonists to multiply for 3 or 4 generations in the presence of a bacteriostatic concentration of drug.

The techniques used for sensitivity testing include qualitative methods—where a primary specimen may be inoculated on to the medium and exhibition of zones of no growth around antibiotic discs is recorded (A.C.P., 1966)—and the semi-quantitative methods of Ericsson (1960) and Bauer *et al.* (1966).

The Ericsson technique (1960) utilises discs placed on standardised media of known volume and depth, in flat-bottomed containers. The inoculum is carefully measured and flood-inoculated on to the surface of the medium. After removal of excess fluid, the antibiotic discs are placed on the medium and a pre-incubation period of diffusion is allowed before the plates are incubated. This procedure ensures that slowly diffusing antibiotics are not handicapped in the presence of rapidly growing organisms. After incubation, the sizes of the inhibition zones are measured and compared with Regression-Slope graphs prepared from testing large numbers of organisms. Reports may then be given in terms of sensitivity/resistance or as M.I.C. values.

The Kirby-Bauer technique (1966) uses Mueller Hinton Agar and discs of high potency. The inoculum is standardised by adjusting the turbidity of an actively-growing broth culture to match that of a barium sulphate standard. Mueller Hinton Agar plates are then inoculated with cotton-wool swabs moistened in this suspension. Barry *et al.* (1970) describe a simpler method of preparation of organisms in which a small loopful of an overnight broth culture is added to molten agar, which is then poured over the surface of the medium. The thin agar-seed layer is said to give better defined zones of inhibition than flooding or surface-streaking methods. After applying sensitivity discs, the plates are incubated immediately or within 30 minutes of preparation. Following overnight incubation at 37° C, zone diameters are measured and compared with the table reproduced overleaf, taken from Bauer (1966) and Ryan *et al.* (1970).

The zone measurements are not reported, only the interpretations Resistant, Sensitive or Intermediate.

Esser & Elefson (1970) found the Kirby-Bauer method to be simple, accurate and reproducible.

Dewees, Poupard & Morton (1970) investigated the effect of storing pre-poured plates of Mueller Hinton Agar and showed that storage up to three weeks at 4° C, in sealed plastic bags, did not appreciably affect the zone sizes of inhibition.

For antibiotic sensitivity studies, Oxoid Mueller Hinton Broth (CM405) is also available.

REFERENCES

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Zone Sizes and their Interpretation for Frequently used Antibacterial Agents.

Anitibotic or Chemotherapeutic Agent	*Disc Potency*	*Inhibition Zone Diameter to Nearest Millimetre*		
		Resistant	*Intermediate*	*Sensitive*
Ampicillin S. aureus	10 µg	20 or less	21–28	29 or more
all other organisms	10 µg	11 or less	12–13	14 or more
Bacitracin	10 units	8 or less	9–12	13 or more
Cephaloridine	30 µg	11 or less	12–15	16 or more
Cephalothin	30 µg	14 or less	15–17	18 or more
Chloramphenicol	30 µg	12 or less	13–17	18 or more
Colistin Sulphate	10 µg	8 or less	9–10	11 or more
Doxycycline Hydrochloride	30 µg	12 or less	13–15	16 or more
Erythromycin	15 µg	13 or less	14–17	18 or more
Gentamicin	10 µg	—	—	13 or more
Kanamycin	30 µg	13 or less	14–17	18 or more
Lincomycin	2 µg	9 or less	10–14	15 or more
Methicillin	5 µg	9 or less	10–13	14 or more
Nalidixic acid	30 µg	13 or less	14–18	19 or more
Neomycin	30 µg	12 or less	13–16	17 or more
Nitrofurantoin	300 µg	14 or less	15–16	17 or more
Novobiocin	30 µg	17 or less	18–21	22 or more
Oleandomycin	15 µg	11 or less	12–16	17 or more
Penicillin-G	10 units	20 or less	21–28	29 or more
Polymyxin-B	300 units	8 or less	9–11	12 or more
Streptomycin	10 µg	11 or less	12–14	15 or more
Sulphonamides	300 µg	12 or less	13–16	17 or more
Tetracycline	30 µg	14 or less	15–18	19 or more
Vancomycin	30 µg	9 or less	10–11	12 or more

Mueller Hinton Broth

Code CM405

FORMULA

Beef, Infusion from	*grams per litre*	**300.0**
Casein hydrolysate (Oxoid L41)		**17.5**
Starch		**1.5**

pH 7.4 (approx.)

DIRECTIONS

Dissolve 21 grams in 1 litre of distilled water. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Oxoid Mueller Hinton Broth has been produced in parallel with Oxoid Mueller Hinton Agar (CM337). Where studies on antibiotic sensitivities are being made both in broth and agar, it will be found to be of particular value to have media of identical nutrient formulation.

Mueller Hinton Agar was developed originally by the authors in 1941 to recover fastidious Neisseria sp. and other organisms. The presence of starch as a protective colloid probably contributes to the value of the medium for the isolation of organisms which may produce toxic materials during their metabolism on the medium or which may have been physiologically damaged (Olsen & Scott, 1946). The broth medium can therefore be used with confidence whenever a highly nutrient liquid medium is required for growth studies.

REFERENCES

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Muller-Kauffmann Tetrathionate Broth Base

Code CM343

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**7.0**
Soya Peptone (Oxoid L44)		**2.3**
Sodium chloride		**2.3**
Calcium carbonate		**25.0**
Sodium thiosulphate		**40.7**
Ox Bile (Oxoid L50)		**4.75**

DIRECTIONS

Suspend 82 grams in 1 litre of distilled water and bring to the boil. Cool below 45° C and add, just prior to use, 19 ml of iodine solution and 9.5 ml of a 0.1% brilliant green solution. Mix well and fill out into sterile tubes or flasks.

Iodine Solution

Iodine	20 grams
Potassium iodide	25 grams
Distilled water to	100 ml

Dissolve the potassium iodide in approximately 5 ml of distilled water, add the iodide and gently warm the solution to completely dissolve it. Make up the volume to 100 ml with distilled water.

Brilliant Green Solution

Brilliant Green (BDH or Chroma)	0.1 grams
Distilled water	100 ml

Add the brilliant green to the distilled water and shake to dissolve the dye. Heat the solution to 100° C for 30 minutes and shake from time to time whilst cooling, to ensure that the dye has completely dissolved. Store in a brown glass bottle or away from light.

SPECIFICATION

The tetrathionate broth of Muller (offered as Oxoid Tetrathionate Broth Base CM29) was modified (Kauffmann, 1935) by the addition of brilliant green, to suppress the growth of *Proteus* species, and ox bile, to improve the isolation of *Salmonellae.*

The brilliant green dye used in the medium has been shown to be critical and Chroma or BDH brands should be used. It is essential that the dye is added as directed because heating the brilliant green or attempting to incorporate it in the basal medium seriously impairs its selective action.

'Oxoid' Tetrathionate Broth Base CM29 and 'Oxoid' Desiccated Ox Bile L50 were used in a large scale investigation between nine laboratories in eight different countries for the preparation of Muller-Kauffmann Tetrathionate Broth (Edel & Kampelmacher, 1969).

'Oxoid' now offer the medium with the Desiccated Ox Bile incorporated into the base.

Incubation of Muller-Kauffmann broth at 43° C was shown to be essential in this trial and the technique used for enrichment of the salmonellae is as follows:

Add approximately 10 grams of sample to 100 ml of Muller-Kauffmann broth. Shake vigorously and immediately place the flasks of medium in a 45° C water-bath for 15 minutes. Remove the flasks from the water-bath, without drying them, and place in an incubator or another water-bath at 43° C.

Subculture the broth after 18–24 hours and again after 48 hours. Take one loopful of broth from the edge of the surface of the fluid and inoculate either two Oxoid Brilliant Green Agar (Modified) CM329 plates (9 cm diameter) without recharging the loop between plates, or one large plate (14 cm diameter).

Incubate the plates at 37° C for 18–24 hours.

REFERENCES

Edel, W. and Kampelmacher, E. H. (1969) 'Salmonella isolation in nine European laboratories using a standardized technique' *Bull. Wld. Hlth. Org.* **41,** 297–306.

Kauffman, F. (1935) 'Weitere erfahrungen mit dem kombinierten Anweicherungsvertahren fur Salmonellabacillen *Ztschr. f. Hyg.* **117,** 26–32.

'Multodisk'

FOR DETERMINING BACTERIAL SENSITIVITY TO ANTIBIOTICS AND SULPHONAMIDES, ETC.

The paper disc method of testing bacterial sensitivity to chemotherapeutic agents is generally accepted (WHO Report No. 210) as the most effective practical means for clinical use. The 'Multodisk' is an extension of the disc principle—each 'Multodisk' is a sterile filter paper disc having six or eight projecting arms. The tip of each arm is impregnated with a different antibacterial agent (identified by a printed code letter) so that it is possible in one application to test the sensitivity of a micro-organism to either 6 or 8 different antibacterial agents. 'Multodisks' are available in a number of different standard combinations, but in order to give complete flexibility to the bacteriologist, 'Multodisks' can be manufactured with virtually any combination of agents required, provided the order is for 20 or more tins.

For full details of technique, interpretation of the test, sensitivity testing media, Standard 'Multodisks' (combinations available from stock) and Agents available for impregnation on Special 'Multodisks', please ask for the separate 'Oxoid' leaflet.

Mycoplasma Agar Base

Code CM401

FORMULA

Bacteriological Peptone (Oxoid L34) *grams per litre*	**10.0**
'Lab-Lemco' Powder (Oxoid L29)	**10.0**
Sodium chloride	**5.0**
Mineral supplement	**0.5**
Agar No. 1 (Oxoid L11)	**10.0**

pH 7.8 (approx.)

Mycoplasma Supplement-G

Code SR59

FORMULA (per vial)

Horse Serum	**20 ml**
Yeast Extract (25% w/v)	**10 ml**
Thallous acetate	**25 mg**
Penicillin	**20,000 units**

Mycoplasma Supplement-P

Code SR60

FORMULA (per vial)

Horse Serum	**6 ml**
Yeast Extract (25% w/v)	**3 ml**
Thallous acetate	**0.008 g**
Glucose	**0.3 g**
Phenol Red	**0.0012 g**
Methylene blue chloride	**0.0003 g**
Penicillin	**12,000 units**
Mycoplasma Broth Base (Oxoid CM403)	**0.146 g**

DIRECTIONS

Add 35.5 g to 1 litre of distilled water. Boil to dissolve the agar and distribute in 80 ml volumes. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C, and add the sterile supplement.

Mycoplasma Supplement—G (Hayflick) Medium

The sterile supplement is prepared by aseptically adding 20 ml of sterile distilled water to the vial and mixing gently. Add this to 80 ml of sterilized Oxoid Mycoplasma Agar or Broth Base (CM 401/403), previously cooled to 50° C.

Bi-phasic medium for *Mycoplasma pneumoniae*

Prepare the sterile supplement by aseptically adding 20 ml sterilized distilled water to the vial and mix gently.

Prepare the solid fraction of the biphasic medium by adding 1 ml of Oxoid Mycoplasma Agar Base (CM401) without supplements to each of ten ¼ oz bijou bottles. Sterilize by autoclaving at 121° C for 15 minutes. Allow to set.

Using aseptic techniques, add 2 ml of the reconstituted selective medium to each agar-containing bijou bottle.

DESCRIPTION

Oxoid Mycoplasma Agar Base (CM401) was formulated as a basal medium to be enriched with any satisfactory supplementary factors used for the growth of mycoplasma (P.P.L.O.).

Edward (1971) stressed the importance of the absence of toxic factors to mycoplasma in the basal medium. Lynn & Morton (1956) paid special attention to the inhibitory factors which can be present in batches of agar. Oxoid Mycoplasma Agar Base contains selected constituents shown to be free from such inhibitory or toxic substances. It also contains a special mineral supplement which improves the growth and colony characteristics of mycoplasma without interfering with the clarity of the medium.

The basal medium should be supplemented with 10% v/v of a 25% w/v extract of baker's yeast (Hayflick, 1965) or Oxoid Yeast Extract (L21) (Lemcke, 1965). The majority of mycoplasma require media enriched with serum; horse serum (20% v/v) is commonly used. Swine or human sera may be substituted for horse serum but the possible presence of antibodies or antibiotics in human serum make media control of great importance (Fallon, 1969). The addition of DNA to the medium to encourage the growth of bovine genital strains and other mycoplasma was suggested by Edward (1954). 20 µg of sodium deoxyribonucleate per ml of medium is quoted by Lemcke (1965).

Antibacterial agents are necessary to prevent overgrowth of the slow-growing mycoplasma by contaminating organisms. Penicillin and thallous acetate are the most common agents used but T-strain mycoplasma are sensitive to thallous acetate. Fallon (1969) states that ampicillin at 1 mg per ml of medium may be substituted for penicillin and thallous acetate.

Penicillin may be used at concentrations between 50 and 500 units per ml and thallous acetate between 1/2000 and 1/8000 (Lemcke, 1965). It is preferable to omit thallous acetate when searching for T-strain mycoplasma (Shepard & Lanceford, 1970).

The two pre-sterilized supplements: Mycoplasma Supplement-G and Mycoplasma Supplement-P have been developed for the improved growth of mycoplasma. Mycoplasma Supplement-G is a general supplement prepared to the formulation of Hayflick (1965), which, when added to Oxoid Mycoplasma Broth or Agar Base produces a complete selective medium for the propagation of sterol-requiring Mycoplasma species of the classical type. Trials carried out in the U.K., by Reference Laboratories in the mycoplasma field have demonstrated the quality and effectiveness of the product.

Mycoplasma Supplement-P is a liquid supplement based on the formulation recommended by the Mycoplasma Reference Laboratory, Colindale, London which is used in conjunction with Mycoplasma Agar Base to form a bi-phasic medium for the isolation and preliminary identification of *Mycoplasma pneumoniae*.

Many species of mycoplasma are aerobes or facultative anaerobes but some prefer micro-aerophilic conditions with the addition of carbon dioxide, or strict anaerobiosis.

Pathogenic strains grow best at temperatures near 37° C while saprophytic strains often grow between 22° and 30°, T-strains have an optimal temperature of 36° C.

Mycoplasma spp. grow best at pH 7.4–8.0 but T-strains prefer pH 6.0–6.5.

Material for cultivation is inoculated into broth or agar medium overlaid with broth. Agar plates are incubated in moist chambers aerobically, anaerobically and in 10% CO_2-90% N_2 atmosphere. Examine the agar surface after 7 days incubation with a dissecting microscope at × 60 magnification, using obliquely transmitted light. The colonies are characteristic with the centre of the colony embedded beneath the surface, giving a 'fried-egg' appearance.

Purification of the organism by further cloning sub-cultures is essential before identification. This may be carried out by removing a plug of agar containing a colony from the plate and using it to inoculate further plates of medium.

Growth inhibition tests using specific antisera may then be carried out (Clyde, 1964).

Bi-phasic medium bottles should be inoculated with a swab or a fleck of sputum and incubated at 37° C for up to three months. Any bottles showing gross turbidity due to growth of bacteria or fungi should be discarded.

Growth of *M. pneumoniae* results in the reduction of Methylene blue followed by production of acid due to the fermentation of glucose, resulting in a colour change of the Phenol red indicator to yellow. Bottles showing such a colour change should be sub-cultured onto agar for further examination. Oxoid Mycoplasma Agar Base supplemented with Oxoid Mycoplasma Supplement–G (SR59) is suitable for this purpose.

REFERENCES

Clyde, W. A. (1964) 'Mycoplasma species identification based upon growth inhibition by specific antisera' *J. Immun.* **92**, 958–962.

Edward, D. G. ff. (1954) 'The Pleuropneumonia Group of Organisms: a Review together with some New Observations' *J. Gen. Microbiol.* **10**, 27–64.

Edward, D. G. ff. (1971) 'Determination of Sterol Requirements for Mycoplasmatales' *J. Gen. Microbiol.* **69**, 205–210.

Fallon, R. J. (1969) 'Isolation Methods for Mycoplasmas from Man and Rodents' in Isolation Methods for Microbiologists. S.A.B. Technical series 3. Academic Press, 41–50.

Hayflick, L. (1965) 'Cell cultures and mycoplasmas' *Texas Rep. Biol. & Med.* **23**, suppl. 1, 285–303.

Lynn, R. J. and Morton, H. E. (1965) 'The Inhibitory Action of Agar on certain strains of Pleuropneumonia-like Organisms' *Appl. Microbiol'* **4**, 339–341.

Shepard, M. C. and Lanceford, C. D. (1970) 'Urease Color Test Medium U-9 for the Detection and Identification of "T" Mycoplasmas in Clinical Material' *Appl. Microbiol.* **20**, 539–543.

Mycoplasma Broth Base

Code CM403

FORMULA

	grams per litre
Bacteriological Peptone (Oxoid L34)	**10.0**
'Lab-Lemco' Powder (Oxoid L29)	**10.0**
Sodium chloride	**5.0**
Mineral supplement	**0.5**

pH 7.8 (approx.)

DIRECTIONS

Dissolve 25.5 grams in 1 litre of distilled water. Mix well and distribute in 80 ml volumes. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C and add the sterile supplement.

DESCRIPTION

Oxoid Mycoplasma Broth Base (CM403) complements Oxoid Mycoplasma Agar Base (CM401).

It requires supplementation with yeast extract, serum and antibiotics, as described for Oxoid Mycoplasma Agar Base.

Carbohydrate fermentation of Mycoplasma can be tested by incorporating 1% w/v carbohydrate and 0.005% w/v phenol red into the broth medium.

A T-strain broth can be prepared by adding to 95 ml broth medium (pH adjusted to 6.0):

Horse Serum	4 ml
Urea	0.05 g
Phenol red	0.001 g
Penicillin	100,000 units

(Shepard & Lanceford, 1970).

A similar medium was described by Taylor-Robinson *et al.* (1971) where reference is made to the use of HEPES buffer to induce large colony forming T-strains.

Taylor-Robinson *et al.* (1971) described the use of broth media for the isolation and titration of viable mycoplasma by the metabolism of arginine or glucose and measuring the pH change in the medium.

A selective medium for *Mycoplasma pneumoniae* was described by Kraybill & Crawford (1965).

Mycoplasma Supplement–G (Oxoid SR59) and Mycoplasma Supplement–P (Oxoid SR60) may be used with this medium as described on pages 198 and 199.

Most strains of mycoplasma are encouraged by growth in a biphasic medium in which a layer of Broth medium covers a basal layer of Mycoplasma Agar. (Reference Mycoplasma Agar Base CM401.)

REFERENCES

Kraybill, W. H. and Crawford, Y. E. (1965) 'A selective medium and colour test for Mycoplasma-pneumoniae' *Proc. Soc. Exp. Biol. Med.* **118**, 965–967.
Shepard and Lanceford (1970) 'Urease Colour Test Medium U-9 for the Detection and Identification of "T" Mycoplasmas in Clinical Material' *Appl. Microbiol.* **20**, 539–543.
Taylor-Robinson, D., Martin-Bourgon, C., Watanable, T. and Addey, J. P. (1971) 'Isolation of T-mycoplasmas from Dogs and Squirrel Monkeys: Biological and Serological Comparison with those Isolated from Man and Cattle' *J. Gen. Microbiol.* **68**, 97–107.

Niacin Assay Medium

Code CM211

FORMULA

	grams per litre
Vitamin-free Casein Hydrolysate	**12.0**
Dextrose	**40.0**
Sodium acetate	**20.0**
l-Cystine	**0.4**
dl-Tryptophane	**0.2**
Adenine sulphate	**0.02**
Guanine hydrochloride	**0.02**
Uracil	**0.02**
Thiamine hydrochloride	**0.0002**
Calcium pantothenate	**0.0002**
Pyridoxine hydrochloride	**0.0004**
Riboflavine	**0.0004**
p-Aminobenzoic acid	**0.0001**
Biotin	**0.0000008**
Dipotassium phosphate	**1.0**
Monopotassium phosphate	**1.0**
Magnesium sulphate	**0.4**
Sodium chloride	**0.02**
Ferrous sulphate	**0.02**
Manganese sulphate	**0.02**

pH 6.8 (approx.)

DIRECTIONS

Suspend 75 grams in 1 litre of distilled water and heat to boiling for 2–3 minutes. Keep the slight precipitate uniformly suspended and dispense 5 ml aliquots. For the assay, adjust the total volume to 10 ml with distilled water after addition of the Test or Control, and autoclave at 115° C for 10 minutes.

DESCRIPTION

A complex medium providing all the growth factors necessary for the microbiological tube assay of niacin (nicotinic acid) and its analogues, using *Lactobacillus plantarum.* The addition of test samples containing niacin to the medium gives a growth response by *Lactobacillus plantarum* (*Lactobacillus arabinosus* 17–5) which may be measured acidimetrically or turbidimetrically (Barton-Wright, 1961).

Oxoid Niacin Assay Medium with added Agar No. 1 (Oxoid L11) and vitamins may also be used for the cup-plate assay of niacin. The cup-plate technique (Barton-Wright, 1962) has been modified by Jones (1961) as follows:

(i) Suspend 18.75 grams of Oxoid Niacin Assay Medium in 250 ml of cold distilled water.

(ii) Add 3.75 grams of Agar No. 1 (Oxoid L11)

(iii) Add 0.5 mg of each of the following: riboflavine, thiamine, pyridoxine, calcium pantothenate, para-aminobenzoic acid.

(iv) Add 0.5 µg of biotin.

(v) Soak for 15 minutes. Mix and steam for 30 minutes to dissolve the agar. Sterilize by autoclaving for 10 minutes at 115° C.

(v) Seed 250 ml of medium at 50–55° C with 12.5 ml of *Lactobacillus arabinosus* suspension. A 50% dilution of the latter should match a Brown opacity tube No. 7.

(vii) Punch holes in the solidified medium (in large assay plates) using a sterile No. 4 cork borer. For standards, add 0.1 ml of a stock solution of niacin containing (a) 2.5 µg per ml and (b) 10 µg per ml.

REFERENCES

Barton-Wright, E. C. (1961) 'Practical Methods for the Microbiological Assay of the Vitamin B-Complex and Amino Acids' *Lab. Prac.* **10**(9), 633–634.
Barton-Wright, E. C. (1962) 'Practical Methods for the Microbiological Assay of the Vitamin B-Complex and Amino Acids' *Lab. Prac.* **11**(3), 199-202.
Jones, Audrey (1961) Personal Communication.

Nutrient Agar

Code—Powder CM3
Tablets CM4

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	**1**
Yeast Extract (Oxoid L20)	**2**
Peptone (Oxoid L37)	**5**
Sodium chloride	**5**
Agar No. 3 (Oxoid L13)	**15**

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 28 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A simple medium, one of the first in the Oxoid range, and one which has repeatedly proved its worth: amongst the many published works which mention the Oxoid medium there are accounts of the cultivation of members of the following genera: *Alysiella, Agrobacterium, Arthrobacter, Bacillus, Brucella, Escherichia, Flavobacterium, Micromonospora, Pseudomonas, Rhizobium, Sarcina, Simonsiella, Staphylococcus, Streptomyces, Thermopolysora, Thiobacillus* and *Xanthomonas.*

Nutrient Agar is an inexpensive medium and so is suitable for teaching and demonstration purposes. It contains a concentration of 1.5% of agar to permit the addition of up to 10% of blood or other biological fluid, as required. The medium, without additions, may be used for the cultivation of organisms which are not exacting in their food requirements.

For a medium which is richer in nutrients, see Blood Agar Base No. 2 CM271.

Nutrient Broth

Code—Powder CM1
Tablets CM2

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	1
Yeast Extract Powder (Oxoid L21)	2
Peptone (Oxoid L37)	5
Sodium chloride	5

pH 7.4 (approx.)

DIRECTIONS

Powder: Add 13 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 10 ml of distilled water, and sterilize by autoclaving at 121° C. for 15 minutes,

DESCRIPTION

A very economical medium for the cultivation of organisms which are not exacting in their food requirements. 500 grams of Oxoid Nutrient Broth powder makes over 38 litres of broth. The reconstituted and sterilized medium may be enriched with other ingredients such as carbohydrates, blood, serum, etc. as required for special purposes. See also Nutrient Broth No. 2 CM67.

Nutrient Broth No. 2

Code—Powder CM67
Tablets CM68

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	**10**
Peptone (Oxoid L37)	**10**
Sodium chloride	**5**

pH 7.5 (approx.)

DIRECTIONS

Powder: Add 25 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This clear medium is comparable to a meat infusion and is richer in nutrients than Nutrient Broth CM1. It gives good growth from small inocula and is recommended for sterility testing for aerobic organisms. Nutrient Broth No. 2 complies with the recommendations in the 'British Pharmacopoeia' (1968) for the composition of a sterility testing medium for aerobes. The medium is ideally suited for subculture, particularly as a secondary growth medium for staphylococci which are to be tested for coagulase production. Nutrient Broth No. 2 made up at double strength corresponds to the medium recommended by the British Standards Institution (1934) for use in the determination of the Rideal-Walker Coefficient of Disinfectants

REFERENCE

British Pharmacopoeia, 1968, 357.
British Standard 541: 1934. 'Determining the Rideal-Walker Coefficient of Disinfectants' BSI, London, p. 9.

Nutrient Gelatin

Code CM135a

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	**3**
Peptone (Oxoid L37)	**5**
Gelatin (Oxoid L8)	**120**

pH 6.8 (approx.)

DIRECTIONS

Suspend 128 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring and cool to below 20° C or leave to set in a refrigerator.

DESCRIPTION

Nutrient Gelatin CM135a, a solid medium at 22° C and below, is employed for the determination of gelatin liquefaction and for the 20° C plate count as described in 'Standard Methods for the Examination of Water and Sewage' (American Public Health Association, 1965). Gelatin is liquefied in a characteristic manner by certain proteolytic organisms; whereas gelatin media have been largely superseded by agar media for most purposes, nutrient gelatin is still employed for differentiation of micro-organisms by their proteolytic effects.

TECHNIQUE

Test for gelatin liquefaction by incubating a Nutrient Gelatin stab or plate culture at 20–22° C. Alternatively, incubate at a higher temperature (usually optimum for the organism under investigation) and then transfer the tube to a refrigerator or into cold water before observation. The latter method not only allows determinations to be carried out on organisms which grow slowly or not at all at 20–22° C but also usually avoids false positive results produced by the release of enzymes after the death of the organisms (Society of American Bacteriologists, 1957). If the medium is incubated at a higher temperature it is necessary to employ uninoculated controls to allow for the hydrolytic effect of heat and other factors. Rates of liquefaction vary considerably, so that some organisms produce liquefaction within a few days whereas others require several weeks. For practical purposes, a maximum incubation period of 14 days is suggested (e.g. American Public Health Association, 1963; Wilson & Miles, 1964). Considerably longer incubation may be necessary—for example Windle Taylor (1958) mentioned some strains of *Klebsiella cloacae* (*Aerobacter cloacae*, Jordon) by which gelatin is liquefied only after 3 months at 20–22° C. Especially where prolonged incubation is necessary, it is important to ensure adequate closure of the containers in order to prevent dehydration of the medium. Besides its presence or absence, the shape and nature of the liquefied portion of the stab culture are often useful identifying characteristics.

Particularly with plate cultures, gelatin liquefaction may be detected sooner by the 'Stone reaction' (Stone, 1935): add a drop of saturated aqueous ammonium sulphate solution, or of fresh 20% aqueous sulphosalicylic acid solution, to an individual colony growing on Nutrient Gelatin. A positive reaction (i.e. gelatin liquefaction) is indicated by the presence of a clear zone round the colony after 10 minutes' contact with either reagent. This method is slightly less sensitive but several strains may be tested on one plate. The 'Stone reaction' is also employed with Staphylococcus Medium No. 110, Code CM145 for the differentiation of staphylococci—see page 252.

Kohn (1953) employed pellets containing formalin-denatured gelatin and charcoal for the demonstration of gelatin liquefaction. These pellets are now available as Oxoid Charcoal Gelatin Discs, Code BR10. They are particularly convenient because they do not melt at any incubation temperature and are used by adding to any culture medium whether liquid, semi-solid or solid. See page 85 for details.

For the standard gelatin plate count on water (American Public Health Association, 1965) dilute the original sample with sterile tap water and place 0.5 or 1.0 ml of the dilutions in each dish of at least two duplicate sets of sterile Petri dishes. Cool the sterile prepared Nutrient Gelatin to approximately 42° C and aseptically add 10 ml to each Petri dish. Mix the contents by tilting and rotation, allow to solidify as soon as possible after pouring and immediately place in an incubator at 19–21° C. Incubate for 48 ± 3 hours and count at least two plates made from the dilution giving between 30 and 300 colonies per plate.

REFERENCES

American Public Health Association (1963) 'Diagnostic Procedures and Reagents' APHA Inc., New York, 4th ed. p. 150 & 294-295.

American Public Health Association, American Waterworks, Water Pollution Control Federation (1966) 'StandardMethods for the Examination of Water and Wastewater' 12th ed., APHA Inc., New York, pp. 596-597.

Kohn, J. (1953) 'A Preliminary Report of a New Gelatin Liquefaction Method' *J. clin. Path.* **6**(3), 249.

Society of American Bacteriologists: Committee on Bacteriological Technic (1957) 'Manual of Microbiological Methods' McGraw-Hill Book Co. Inc., London, pp. 158–159.

Stone, R. V. (1935) 'A Cultural Method for Classifying Staphylococci as of the "Food Poisoning" Type' *Proc. Soc. Exper Biol Med.* **33**, 185–187.

Wilson, G. S. and Miles, A. A. (1964) 'Topley and Wilson's Principles of Bacteriology and Immunity' 5th ed., Vol. 2, Edward Arnold Ltd., London.

Windle Taylor, E. (1958) 'The Examination of Waters and Water Supplies' 7th ed., Churchill Ltd., London, pp. 414 and 422.

Optochin Discs

Code DD1

DESCRIPTION

Bowers and Jeffries (1955) have shown that there is complete correlation between bile-solubility and full 'Optochin' sensitivity for the differentiation of *Diplococcus pneumoniae* from streptococci. Oxoid 'Optochin' Discs are paper discs ready impregnated with 'Optochin' (ethylhydrocuprein hydrochloride) which provide a convenient and reliable alternative to the bile-solubility test. Pneumococci are sensitive to 'Optochin' so that a culture shows a zone of inhibition around the impregnated disc, whilst streptococci either grow right up to the edge of the disc or, occasionally, show a very small zone of inhibition.

TECHNIQUE

Streak a pure culture of the organism to be tested across one half of a Blood Agar plate (Blood Agar Base CM55 with 7% of sterile blood) and apply an 'Optochin' Disc immediately before incubation. At the same time apply a second 'Optochin' Disc to the other half of the plate, previously streaked with a known pneumococcus, in order to provide a positive control. After incubation, pneumococci show a zone of inhibition at least 5 mm from the edge of the disc, whilst streptococci are completely resistant or show a small zone of inhibition extending not more than 2 mm from the edge of the disc.

Oxoid 'Optochin' Discs may also be placed on the primary culture plate, before incubation, in order to provide rapid indication of the presence of large numbers of pneumococci.

PLEASE NOTE

For DIAGNOSTIC purposes Optochin (Code OP) can be included in Special Multodisk combinations if required.

REFERENCE

Bowers, E. F. and Jeffries, L. R. (1955) 'Optochin in the Identification of *Str. pneumoniae* *J. clin. Path,* **8**, 58–60.

Ox-Bile Desiccated

Code L50

Dehydrated, purified fresh bile (i.e. ox-gall) which is used as a selectively inhibitory agent in bile media, such as Brilliant Green Bile (2%) Broth CM31. Ox-Bile Desiccated is prepared by a low temperature dehydration process which ensures a uniform product and eliminates the difficulties encountered in obtaining satisfactory supplies of fresh bile. An 8 to 10% solution of the dehydrated product is equivalent to fresh bile.

Ox-Cell Antigen

Code BR9

A 20% suspension of washed red cells in physiological saline, heated and preserved with 0.5% phenol. Ox-Cell Antigen is a laboratory tested product for use with Guinea-Pig Kidney Antigen (see page 110) and a 2% suspension of sheep red cells in the Paul-Bunnell reaction for the diagnosis of infective mononucleosis. The sheep cells should be more than one day and less than seven days old.

Barrett's technique for the performance of this absorption test, (Barrett, 1941) has been modified, see Cruickshank (1968).

Store at 4° C.

REFERENCES

Barrett, A. M. (1941) 'The Serological Diagnosis of Glandular Fever (Infectious Mononucleosis): A New Technique' *J. Hyg.* Camb. **41**, 330. Medical Revertrology.
Cruickshank, R. (1968) 11th ed., Livingstone Ltd., Edinburgh and London, p. 914–916.

Ox-Heart Desiccated

Code L60

Ox-Heart Desiccated is specially manufactured for the preparation of infusion media. Obtained by the desiccation of fresh ox-heart under conditions controlled to ensure maximum retention of nutritive properties, the dehydrated product is equivalent to five times its weight of fresh heart tissue.

To prepare a heart infusion medium, add 50 grams to 1 litre of distilled water and allow to infuse for 1 hour at 50° C. Boil for a few minutes to coagulate protein, then filter and add 10 grams of Peptone (Oxoid L37) and 5 grams of sodium chloride. Adjust the reaction of the medium at pH 7.2, bring to the boil and refilter before sterilization by autoclaving to 121° C for 15 minutes.

Peptones and Hydrolysates

Nägeli has been credited as the first bacteriologist to discover that chemo-organotrophic organisms grow best in culture media containing a partially digested protein material. His papers published in 1880 and 1882 described this material as 'peptone'.

Peptone is a chemically indefinite term used to describe the water-soluble products obtained after hydrolysis of proteins. It contains a mixture of free amino-acids and polymers of amino-acids (peptides) in increasing numbers to the largest size of peptide (proteoses) which can remain in solution after heating to 100° C.

Peptones are manufactured by the use of enzymes or strong mineral acids to hydrolyse proteins. The problems associated with the production of peptones were quickly recognised and the manufacture of peptone became the concern of commercial suppliers. Peptone, in fact, was the first complex culture medium ingredient supplied by Industry and was the forerunner of the large range of commercial culture media now available.

Oxoid commenced its investigation into the manufacture of peptone in 1924.

The variety of peptones produced reflect the differing demands of micro-organisms for amino-acids and peptides. The proteins used for peptone manufacture may be divided into meat proteins (including casein and gelatin) and plant proteins (soya protein being the outstanding example). The peptones produced from these various proteins may be further sub-divided into those produced by acids, or by enzymes such as pepsin, trypsin or papain.

The differing enzymes break the peptide bonds in the protein molecule at different places, thus releasing a variety of peptides and free amino-acids. Whereas acid-hydrolysis eventually breaks every peptide bond and produces free amino-acids only. Unfortunately it also destroys some valuable amino-acids, such as tryptophane.

Amino-acids may be used by micro-organisms as 'building-bricks' for protein construction or they may be utilised as a source of energy, forming compounds such as hydrogen sulphide, indole, amines, etc. In diagnostic tests, peptones may be selected which have predominant virtues. Thus peptones rich in tryptophan are selected for indole tests. Peptones with high sulphur content may be used for hydrogen sulphide tests.

In the large-scale growth of micro-organisms either the organisms themselves are harvested (for vaccine production) or the metabolites of the organisms are retained (such as antibiotics or exo-toxins). Special peptones are required for these purposes and constant research goes on to produce peptones yielding higher numbers of organisms or greater concentrations of metabolite.

Peptones donate other factors than amino-acids to culture media. Nucleic acid fractions, minerals and vitamins are accessory substances supplied. Plant peptones will also provide carbohydrates which can stimulate the growth of micro-organisms.

It is the presence of alkaline earth metals and phosphates in peptone that causes precipitates to appear when the pH is adjusted towards neutrality. Hence the advantage of purchasing peptones that are manufactured at a neutral pH value.

Casein Hydrolysate (Acid)

Code L41

A technical hydrolysate prepared by the hydrolysis of casein with hydrochloric acid, followed by neutralization with sodium carbonate. Much of the resultant sodium chloride is removed by a special process. Casein Hydrolysate (Acid) provides the necessary nitrogenous material for culture media or may be used when amino-acid mixtures are specified.

Oxoid Casein Hydrolysate (Acid) has found a variety of applications including use in growth studies concerned with submerged and continuous culture.

Lactalbumin Hydrolysate

Code L48

A peptone prepared by the pancreatic digest of the lactalbumin protein obtained from milk. The high levels of essential amino-acids make it a valuable supplement in tissue culture media. It will also serve as a highly nutritious substrate for many micro-organisms, including the lactobacilli.

Liver Digest Neutralised

Code L27

A biologically standardized papain digest of ox liver, for use as a source of nutrients in microbiological culture media. Liver Digest provides essential growth factors and amino-acids for the cultivation of *Trichomonas vaginalis* and other fastidious protozoa, pathogenic and saprophytic fungi, bacteria, and pleuropneumonia-like organisms (PPLO).

The digest is water soluble and compatible with other culture media ingredients, and may be sterilized by filtration or by autoclaving; thus it is suitable for use as an integral part of many culture media or as a valuable supplement. It often may be used to replace more costly nutrient sources, e.g., in some culture media Liver Digest may be used as an alternative to liver infusion, peptones, yeast extract, or heated blood.

As a general guide, Liver Digest will replace peptone weight for weight.

Liver Digest is an essential constituent of Trichomonas Medium CM161 (Feinberg and Whittington, 1957—also see page 266) which is employed for the cultivation and detection of *Trichomonas vaginalis* and *Candida* species.

Feinberg, J. G. and Whittington, Joan M. (1957) 'A culture medium for *Trichomonas vaginalis Donné* and species of *Candida*' *J. clin. Path.* **10**(4), 327–329.

Peptone Mycological

Code L40

An established peptone for the cultivation of dermatophytes, saprophytic moulds and yeasts.

The question of nomenclature of the pathogenic fungi, hitherto somewhat confused, has been largely clarified by the publication by the Medical Research Council (1949). This confusion was due to slight morphological differences, probably caused by variations in the nutritional value of the media, causing variants of the same organisms to be wrongly assigned to different species. It is therefore of paramount importance, particularly in diagnostic work, to employ a standard medium incorporating a constant nutrient peptone. This has been stressed by Hodges (1928) who demonstrated that variation in the macroscopic appearance of cultures of the same organism is governed by the peptone used. Carlier (1948) showed that a satisfactory medium could be prepared from peptone, glucose and agar, and that this could be employed as a serviceable substitute for the standard American medium of Hodges.

With the above considerations in mind, Mycological Peptone was developed specifically for incorporation in solid media used for the isolation and diagnosis of pathogenic and non-pathogenic fungi. It rapidly gives a luxuriant growth with typical morphology and pigmentation. Since it does not encourage bacterial growth because of its acidity, media containing this peptone are useful for the isolation of pathogenic fungi from material heavily infected with bacteria.

Carlier, Gwendoline I. M. (1948) 'An all-British mycological culture medium' *Brit. J. Derm. Syph.* **60**, 61–63.
Hodges, R. S. (1928) 'Cultures of ringworm fungi on Sabouraud's proof mediums and on mediums prepared with American peptones and sugars' *Arch. Derm. Syph.* **18**, 852–856.
Medical Research Council (1949) Memo No. 23: 'Nomenclature of fungi pathogenic to man and animals' HMSO, London.

Peptone Bacteriological

Code L37

An all-purpose peptone with a wide range of applications in routine, diagnostic and research bacteriology. Apart from the fact that L37 is a constituent of many Oxoid media, there are many published references to its use as an ingredient of media for the investigation of: *Escherichia coli* and coliforms, *Bacillus* species, *Staphylococcus aureus* and other *Micrococcaceae*, *Aerobacter* and *Klebsiella* species, streptococci, clostridia, plant pathogens, *Vibrio*, *Proteus*, *Pseudomonas*, *Nocardia*, *Corynebacterium*, *Lactobacillus*, *Leptotrichia*, *Photobacterium*, *Salmonella* and *Spirillum* species, *Bordetella pertussis*, *Serratia marcescens*, a few fungi, and even a flagellate (*Strigomonas*).

Peptone Bacteriological Neutralised

Code L34

Oxoid Peptone Bacteriological Neutralised is a very nutritious, all-purpose peptone prepared by the enzymatic digestion of selected animal proteins. It was specially prepared for use with other refined culture media ingredients, with which it can be mixed in any proportion to give a solution free from haze, cloudiness or precipitation. This peptone has a wide range of applications in routine diagnostic and research bacteriology. It may be used with confidence wherever a high quality bacteriological peptone is called for.

Peptone P

Code L49

A pepsin digest of fresh meat, for use in bacteriological culture media, which complies with the U.S.P. specification (1) for peptic digest of animal tissue.

Peptone P is a constituent of Sabouraud Liquid Medium CM147 (see Oxoid Manual) which is the equivalent of the sterility test medium described in the U.S. Pharmacopeia, 1965 (2). This peptone may be incorporated in a variety of liquid and solid media, including those for the determination of haemolytic reactions. Due to its high sulphur content, Peptone P is also recommended as a constituent of media for hydrogen sulphide formation.

United States Pharmacopeia, 17th revision, 1965, (1) pp. 830 and 831, (2) pp. 1015 and 1016.

Peptonized Milk

Code L32

A peptone for use in media for the isolation and cultivation of *Lactobacillus* species, and the bacteriological examination of dairy products. Peptonized Milk consists of a water-soluble powder prepared by the pancreatic digestion of high grade skim milk powder. This preparation constitutes a source of more readily available nitrogen than milk or milk powder. Peptonized Milk can be used (as a simple solution, solidified with agar only, or as a constituent of more complex media) for the investigation of lactic acid bacteria such as *Lactobacillus acidophilus* and *Lactobacillus bifidus*.

Proteose Peptone

Code L46

A specialized peptone, prepared by the enzymatic digestion of selected fresh meat, for use in media for the production of bacterial toxins. Proteose peptone is especially suitable in media for *Corynebacterium diphtheriae* toxin, including that for the Elek reaction for the recognition of toxigenic strains, as well as in media for the production of toxins from staphylococci, clostridia and salmonellae. Media incorporating this highly nutritious peptone are suitable for the cultivation of different bacteria with a wide range of nutritional requirements, e.g. *Neisseria*, *Staphylococcus*, *Haemophilus*, *Salmonella*, *Pasteurella*, *Corynebacterium* and *Histoplasma* species. Proteose Peptone may also be successfully employed in media for special biological tests such as the methyl-red and Voges-Proskauer reactions.

Special Peptone

Code L72

A specially prepared mixture of peptones, including meat, plant and yeast digests, designed to encourage the growth of the most demanding organisms. It contains the widest spectrum of peptide sizes available in any peptone, together with those minerals, vitamins, nucleotides and other carbon compounds present in the individual peptones.

Peptone Soya Neutralised

Code L44

Oxoid Soya Peptone is a vegetable peptone, prepared by the enzymatic digestion of soya bean meal, for use in media for the cultivation of micro-organisms, including fungi. It has a high carbohydrate content and so is suitable for many purposes other than fermentation studies. Media containing this peptone are employed for the cultivation of many fastidious organisms, where rapid and profuse growth is required, and for microbiological assay procedures.

Average Analyses of Oxoid Peptones

	Casein Hydrolysate (Acid) L41	*Lactalbumin Hydrolysate* L48	*Liver Digest* L27	*Peptone Mycological* L40	*Peptone Bacteriological* L37	*Peptone Bacteriological Neutralised* L34	*Peptone P* L49	*Peptonised Milk* L32	*Proteose Peptone* L46	*Special Peptone* L72	*Peptone Soya* L44	*Tryptone* L42	*Tryptone T* L43	*Tryptose* L47
General (% *w/w*)														
Moisture	3.0	5.9	5.2	4.4	4.4	3.8	3.2	5.4	6.0	3.5	4.1	3.6	3.6	4.7
Ash	36.6	5.5	13.1	3.8	6.7	11.3	16.3	9.4	9.5	12.1	13.7	8.1	13.9	12.4
Chloride (as NaCl)	29.0	1.8	4.6	0.6	2.3	5.5	10.3	2.0	4.3	4.6	3.3	0.4	4.8	5.7
Phosphate (as P_2O_5)	1.3	1.4	2.3	1.5	1.3	1.2	0.7	2.7	1.6	1.7	0.7	1.9	2.5	1.2
Total Nitrogen	8.3	12.5	11.4	13.6	14.3	13.3	12.8	5.7	13.6	12.4	9.3	13.2	12.7	12.7
Amino Nitrogen	4.8	6.1	3.2	3.4	2.5	2.3	2.8	1.7	3.6	3.9	2.2	4.8	3.7	3.7
Amino N/Total N	57.8	49.0	28.1	25.0	17.5	17.3	21.9	29.8	26.4	31.5	23.7	36.4	29.1	29.1
Lipids	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Ammonia	0.62	1.29	0.92	1.13	0.70	0.47	0.99	0.43	0.92	0.66	0.96	0.75	0.97	0.84
Lactose (by difference)	—	—	—	—	—	—	—	43.4	—	—	—	—	—	—
Carbohydrate (as dextrose)	—	—	—	—	—	—	—	—	—	—	13.9	—	—	—
pH of 2% sol. (after autoclaving)	7.0	6.3	7.0	5.4	6.2	7.0	7.0	5.4	7.0	7.0	7.0	7.0	7.0	7.0
Amino Acids % *w/w*														
Alanine	1.64	4.58	3.42	5.15	6.08	5.00	6.94	1.93	4.95	3.72	2.52	2.6	2.20	3.53
Arginine	1.66	3.07	3.43	4.45	5.43	3.69	6.24	0.40	3.43	3.16	5.03	3.1	0.83	2.71
Aspartic Acid	4.04	10.49	5.07	6.79	8.29	7.65	8.28	2.78	6.82	6.47	7.94	6.8	4.72	6.15
Cystine	0.13	2.60	0.84	0.67	0.46	0.48	0.33	0.17	0.59	0.53	0.80	0.3	0.17	0.40
Glutamic Acid	15.29	15.94	9.48	12.14	8.76	12.59	14.62	12.49	12.29	16.91	14.68	17.3	17.32	15.37
Glycine	1.09	1.84	4.62	5.98	5.55	8.20	15.84	0.90	7.59	3.19	2.98	1.8	1.36	4.49
Histidine	0.42	1.78	1.53	1.81	1.66	1.51	1.41	1.22	1.53	1.88	1.70	2.1	1.65	1.68
Isoleucine	2.50	4.97	2.15	2.83	2.76	2.50	2.46	2.11	2.15	3.47	2.50	3.8	3.65	2.72
Leucine	4.33	10.70	4.70	6.03	5.83	4.46	5.13	3.76	4.70	6.04	4.46	6.9	6.42	5.05
Lysine	5.14	11.25	4.52	6.11	6.60	4.67	4.82	2.53	5.65	5.52	3.92	7.9	4.90	6.17
Methionine	1.85	1.63	1.22	1.68	1.71	1.11	1.36	0.95	1.22	1.88	0.55	1.8	1.44	1.22
Phenylalanine	2.89	3.08	2.55	3.26	2.64	2.98	2.75	1.82	2.55	3.18	3.01	3.6	3.49	2.83
Proline	5.54	4.06	2.87	4.61	6.90	5.54	7.47	4.10	4.36	7.43	3.64	8.5	6.79	5.19
Serine	0.93	1.70	0.75	0.73	2.28	2.03	1.46	1.11	0.68	2.38	0.68	1.7	0.88	0.86
Threonine	1.47	3.24	1.41	1.87	2.59	1.86	1.95	1.25	1.70	2.36	1.38	2.3	1.69	1.66
Tryptophan	—	1.32	0.80	0.72	0.52	0.53	0.33	0.55	0.61	0.68	0.69	1.0	1.11	0.86
Tyrosine	2.08	0.67	2.31	2.01	2.31	1.95	1.95	0.84	2.13	1.07	2.36	1.4	2.16	1.78
Valine	3.54	3.92	3.33	4.36	3.96	3.40	3.70	2.92	3.04	4.59	3.10	5.1	4.94	3.75
Metals (*w/w*)														
Calcium	243 ppm	5100 ppm	1800 ppm	2300 ppm	1330 ppm	143 ppm	109 ppm	1.35%	980 ppm	2000 ppm	930 ppm	3460 ppm	87 ppm	2220 ppm
Copper	1 ppm	1.3 ppm	49 ppm	13 ppm	2 ppm	1.1 ppm	1.6 ppm	4.2 ppm	3.5 ppm	5.6 ppm	3.7 ppm	1.0 ppm	1.4 ppm	2.25 ppm
Iron	56 ppm	110 ppm	405 ppm	210 ppm	71 ppm	79 ppm	31 ppm	102 ppm	68 ppm	97 ppm	207 ppm	69 ppm	22 ppm	68 ppm
Lead	<2 ppm	3.9 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm	<2 ppm
Magnesium	143 ppm	480 ppm	1050 ppm	1230 ppm	790 ppm	200 ppm	98 ppm	1600 ppm	1130 ppm	710 ppm	2630 ppm	283 ppm	26 ppm	706 ppm
Manganese	0.3 ppm	3.9 ppm	0.2 ppm	0.2 ppm	0.2 ppm	0.2 ppm	0.2 ppm	0.2 ppm	0.2 ppm	0.2 ppm	0.2 ppm	0.3 ppm	<0.2 ppm	0.2 ppm
Potassium	0.27%	0.85%	1.45%	1.17%	1.23%	1.23%	1.43%	1.30%	1.46%	1.10%	2.90%	0.20%	1.40%	0.83%
Sodium	13.5%	0.92%	0.40%	0.58%	1.60%	2.46%	5.16%	2.10%	1.34%	4.40%	1.10%	3.20%	4.50%	2.27%
Tin	<20 ppm	86 ppm	<20 ppm	< 20 ppm	<20 ppm	<20 ppm	<20 ppm	<20 ppm	<20 ppm	<20 ppm	<20 ppm	<20 ppm	<20 ppm	<20 ppm
Zinc	25 ppm	9.8 ppm	120 ppm	70 ppm	26 ppm	27 ppm	15 ppm	62 ppm	56 ppm	69 ppm	27 ppm	49 ppm	36 ppm	53 ppm

The analysis of materials containing soluble degradation products of proteins presents considerable difficulties in the interpretation of results. Because of the overlap of nitrogen containing fractions such as primary and secondary proteoses, peptones, polypeptides, and the non-specific nature of most analytical techniques—the results obtained are highly empirical and mean very little unless the nature of the sample and the method of analysis is known. For this reason the methods of nitrogen fractionation analysis, used to obtain the figures given in the table, are shown overleaf:

Tryptone

Code L42

Tryptone is a tryptic hydrolysate of casein. It can be used in any formula where a pancreatic or tryptic digest of casein is specified, and complies with the specification for pancreatic digest of casein in the U.S. Pharmacopeia, 1965. An extremely rich source of amino-acid nitrogen, this peptone may be recommended for the preparation of sterility test media, Dubos medium, and (due to a high tryptophan content) media for indole production. Tryptone is especially suitable for inclusion in many media used by the canning industry; an example of this is dextrose tryptone agar, which is used for the detection of 'flat-sour' thermophiles (see page 111). The hydrolysate is often mentioned in published works, either as a constituent of culture media for metabolic or growth studies or for other purposes where high performance and uniformity of composition are of paramount importance.

United States Pharmacopeia, 17th revision, 1965, pp. 1013–1014.

Total Nitrogen
By Kjeldahl's method using copper and sodium sulphates, with screened methyl red as the indicator.

Total Proteose Nitrogen
By precipitation with saturated (at 40° C.) zinc sulphate solution, after removal of acid-insoluble and coagulable solids, and determination of the precipitated proteose nitrogen by Kjeldahl's method.

Primary Proteose Nitrogen
As with Total Proteose Nitrogen, but half-saturated zinc sulphate solution is employed.

Amino-acid Nitrogen
Amino-nitrogen associated with a free acidic group is determined by potentiometric titration using a formaldehyde method (i.e., formol titration).

Tryptone T

Code L43

Oxoid Tryptone T was especially developed from Oxoid Tryptone (Code L42) and is particularly suitable for inclusion in culture media for production of toxin by *Clostridium tetani*, or for use in media where a high toxin yield is required. The digest is produced from high grade casein by controlled enzymatic hydrolysis, and purifed by the method of Mueller and Miller (1954).

Mueller and Miller found that, if unpurified pancreatic digests of casein were employed in media for tetanus toxin production, there were occasional variations in toxin yields due to inhibitory substances. This problem was solved by treatment with calcium phosphate, which effectively removed inhibitors of toxin production.

Mueller, J. H. and Miller, Pauline A. (1954) 'Variable factors influencing the production of tetanus toxin' *J. Bact.* **67**, 271–277.

Tryptose

Code L47

Oxoid Tryptose is a mixed peptone with unique nutritional properties rendering it suitable for use in media for the isolation and cultivation of *Brucella* species, streptococci and other fastidious organisms. Its highly nutritious composition makes it suitable for use in media for the isolation of the more fastidious bacteria and where rapid or profuse growth is required, e.g. in blood culture. Oxoid Tryptose is also recommended for use in blood agar and other media for the determination of haemolytic reactions, and in media for indole production.

Peptone Water

Code—Powder CM9
Tablets CM10

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10**
Sodium chloride		**5**

pH 7.2 (approx.)

DIRECTIONS

Powder: Add 15 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 121° C for 15 minutes.

When sterile solutions are to be added after autoclaving, reduce the volume of water for reconstitution by an equal amount.

DESCRIPTION

Peptone Water may be used as a growth medium or as the basis of carbohydrate fermentation media, whilst a pure culture in Peptone Water is a convenient inoculum for a series of fermentation tubes or other diagnostic media.

Sterile solutions of carbohydrates and other reagents are available for addition to this medium.

Peptone Water, adjusted to pH 8.4, is suitable for the cultivation and enrichment of *Vibrio comma* from infected material (Cruickshank, R. 1968).

The medium was formerly used for the performance of the indole test, but now better results can be obtained by the use of Tryptone Water CM87 (see page 274).

This medium is also available with added Andrade indicator (Andrade Peptone Water CM61) or with added phenol red indicator (Phenol Red Peptone Water CM63). The Andrade medium changes from colourless to pink if there is acid production. The phenol red medium is orange when neutral, yellow when acid, and deep red when alkaline. Both media are prepared and sterilized in the same manner as with ordinary peptone water except that an inverted Durham tube is included for the indication of gas production. If carbohydrate solutions are added, before or after autoclaving, the volume of water used for reconstitution should be reduced by an equivalent amount. The Andrade medium is pink when hot but becomes colourless again on cooling.

REFERENCE

Cruickshank, R. (1968) 'Medical Microbiology' 11th ed., Livingstone Ltd.' London, p.268.

Petri Dishes—Sterile Disposable Plastic

Code DP2

The use of Disposable Plastic Petri Dishes has gained wide acceptance in many laboratories. The fact that they are sterile, light in weight, cheap and easily disposable has enabled the technician to dispense with a significant portion of the laborious preparation which has hitherto been accepted as a necessary part of laboratory practice. The advantage of having a stock of sterile dishes always available at short notice will be obvious to those in a busy laboratory.

DESCRIPTION

The Oxoid dish, made to an exacting specification, fulfils the requirements of the most critical worker. Manufactured from crystal clear polystyrene, it is sterile, clean and biologically inert. It has no adverse effect on culture media or on bacterial growth.

DIMENSIONS

Dish—90 mm (3.5 in.) × 15 mm (0.6 in.).
Lid—95 mm (3.75 in.) × 7.5 mm (0.3 in.).

ADVANTAGES

Extensive use in a variety of laboratories, both large and small, has revealed many advantages over conventional dishes:

(i) They are inexpensive.

(ii) The tedious and costly work of preparing a scrupulously clean and sterile dish is eliminated.

(iii) The size and weight of the dish makes for easier handling.

(iv) If accidentally dropped, breakage does not usually occur and the agar is unlikely to become detached.

(v) Being crystal clear, they are ideally suitable for plate counts.

(vi) They are easy to write on.

(vii) Because the base and the lids are slightly recessed, stacking is facilitated.

(viii) The dish, once used, is easily disposed of.

METHODS OF DISPOSAL

After use, dishes may be discarded directly into the incinerator, provided that its temperature is high and that suitable precautions are taken during handling. The dishes are fully combustible, leaving no glue-like residue.

Alternatively, they may be placed in a suitable container, autoclaved, and thrown away or preferably incinerated.

PRESENTATION

Oxoid Disposable Plastic Petri Dishes are presented in polythene bags each containing 10 sterile dishes. For details of price and packing, please ask for current leaflet.

Phenolphthalein Phosphate 1%

Code SR31

There is a high degree of correlation between phosphatase production and coagulase production as indicators of staphylococcal pathogenicity—thus a staphylococcus which will liberate phenolphthalein from phenolphthalein diphosphate will almost certainly coagulate blood plasma and be pathogenic to man. Phosphatase producers are detected by cultivation on a nutrient agar containing 0.01% of the phosphate: when exposed to ammonia vapour, their colonies become bright pink (Barber and Kuper, 1951).

Phenolphthalein phosphate should not be incorporated in liquid media. The sterile 1% solution is available in 1 ml ampoules packed in boxes of ten.

TECHNIQUE

Prepare and sterilize 100 ml of Blood Agar Base CM55. Cool to 50° C and aseptically add 1 ml of Oxoid Phenolphthalein Phosphate 1%. Mix. Pour plates, allow to solidify, and inoculate in the usual way. Incubate overnight at 37° C and then expose the plate to ammonia vapour. Phosphatase-positive colonies become bright pink.

REFERENCE

Barber, M. and Kuper, S. W. A. (1951) 'Identification of *Staphylococcus pyogenes* by the Phosphatase Reaction' *J. Path. Bact.* **63**, 65–68.

Phenol Red Peptone Water

See page 217.

Phenylalanine Agar

Code CM297

FORMULA

	grams per litre
Yeast Extract Powder (Oxoid L21)	**3**
dl-Phenylalanine	**2**
Disodium phosphate	**1**
Sodium chloride	**5**
Agar No. 1 (Oxoid L11)	**12**

pH 7.3 (approx.)

DIRECTIONS

Suspend 23 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense into tubes or bottles and sterilize by autoclaving at 121° C for 15 minutes. Solidify in a sloping position.

DESCRIPTION

Phenylalanine Agar, a modification of that described by Ewing, Davis and Reavis (1957), is recommended for the detection of phenylalanine deaminase systems in the differentiation of *Proteus* and *Providence* organisms from other Enterobacteriaceae.

The test depends on the conversion of phenylalanine to phenylpyruvic acid by oxidative deamination. The phenylpyruvic acid can readily be identified by the application of ferric chloride, resulting in a green coloration for a positive reaction.

The production of phenylpyruvic acid from phenylalanine, as a means of bacterial identification, was first employed by Henriksen & Closs (1938). Subsequent work by Henriksen (1950), Buttiaux *et al.* (1954) and Singer & Volcani (1955) has shown the reaction to be almost specific for *Proteus* and *Providence* organisms.

This test, together with urease activity, is now regarded as an important diagnostic factor in the identification of *Proteus* species.

TECHNIQUE

The method described in the International Association of Microbiological Societies Report (1958) is as follows:

(1) Heavily inoculate a phenylalanine slope.

(2) Incubate overnight.

(3) Run 0.2 ml of a 10% ferric chloride solution over the surface of the slope.

A positive reaction is indicated by the appearance of a green coloration on the slope and in the free fluid at its base within 1–5 minutes.

REFERENCES

Butti aux,R., Osteux, R., Fresnoy, R. and Monamezi, J. (1954) 'Les propriétés biochemiques' caractéristiques du genre Proteus. Inclusion souhaitable des Providencia dans celui-ci' *Ann. Inst. Pasteur* **87**, 375–386.
Ewing, W. H., Davis, B. R. and Reavis, R. W. (1957) 'Phenylalanine and Malonate media and their use in enteric bacteriology' *Publ. Hlth. Lab.* **15**, 153.
Henriksen, S. D. (1950) 'A comparison of the phenylpyruvic acid reaction and the urease test in the differentiation of Proteus species from other enteric organisms' *J. Bact.* **60**, 225–231.
Henriksen, S. D. and Closs, K. (1938) 'The production of phenylpyruvic acid by bacteria' *Acta path. microbiol. Scand.* **15**, 101–113.
Report of the Enterobacteriaceae Subcommittee of the Nomenclature Committee of the International Association of Microbiological Societies (1958) *Int. Bull. bact. Nom. Tax* **8**, 25.
Singer, J. and Volcani, B. E. (1955) 'An improved ferric chloride test for differentiating Proteus-Providence group from other Enterobacteriaceae' *J. Bact.* **69**, 303–306.

Phosphate Buffered Saline (DULBECCO)

Code BR14a (Dulbecco 'A' Tablets)

FORMULA

Sodium chloride	*grams per litre*	**8.0**
Potassium chloride		**0.2**
Disodium hydrogen phosphate		**1.15**
Potassium dihydrogen phosphate		**0.2**

pH 7.3

DIRECTIONS

Dissolve 10 tablets in 1 litre of distilled water and autoclave for 10 minutes at 115° C. The solution will be quite free from insoluble matter.

Code SR39 (Dulbecco 'B' Ampoules)

FORMULA

Calcium chloride	*grams per litre*	**0.1**
Magnesium chloride		**0.1**

and distilled water to 5 ml

DIRECTIONS

Add one 5 ml ampoule of Dulbecco 'B' Solution to 1 litre of Dulbecco 'A' Solution to make the complete Dulbecco Phosphate Buffered Saline pH 7.4.

DESCRIPTION

This balanced salt solution is issued in divided form to meet the requirements of those tissue culture workers who use the Dulbecco Solution with and without calcium and magnesium. Oxoid Dulbecco 'A' Solution corresponds to the original formulation of Dulbecco and Vogt (1954) except that calcium and magnesium are omitted and may be added separately as Dulbecco 'B' Solution SR39.

Solution 'A' and Solution 'A + B' are extensively used as diluents during tissue culture procedures. An example of the use of Solution 'A' is in the suspension of cells from a monolayer culture (Paul, 1965). Cells growing in monolayer on a glass surface may be brought into suspension by a number of methods, including the application of chelating agents such as Versene (diamino-ethane-tetra-acetic acid) or its salts. Chelating agents bind divalent cations, such as calcium and magnesium ions, and it is probably this effect which causes the cells to become detached from the glass. Dulbecco 'A' Solution is therefore used to rinse the sheet of cells free from growth medium before application of the chelating agent—this latter solution may consist of Dulbecco 'A' Solution containing 0.02% w/v of sodium versenate and 0.02% w/v of dextrose.

REFERENCES

Dulbecco and Vogt (1954) *J. Exp. Med.* **99**(2), 167–182.
Paul, J. (1965) 'Cell and Tissue Culture' 3rd ed., Livingstone Ltd., London.

P.K.U. Test Agar

Code CM365

FORMULA

Dextrose	*grams per litre*	5.0
Di-potassium phosphate		15.0
Mono-potassium phosphate		5.0
Ammonium chloride		2.5
Ammonium nitrate		0.5
Sodium sulphate		0.5
L-Alanine		0.25
L-Glutamic acid		0.50
L-Asparagine		0.50
Magnesium sulphate $7H_2O$		0.05
Manganese chloride $4H_2O$		0.005
Ferric chloride $6H_2O$		0.005
Calcium chloride		0.0025
Agar No. 1 (Oxoid L11)		15.0

pH 6.8 (approx.)

DIRECTIONS

Suspend 45 grams in 1 litre of distilled water at 60° C and add β-2-thienylalanine. Boil to dissolve the medium completely. Cool to 50° C and add the *Bacillus subtilis* spore suspension. Mix thoroughly and pour plates. If desired the medium can be sterilized by autoclaving at 121° C for 15 minutes. Guthrie and Susi (1963) give details of the preparation and of the amounts of reagents used in this test.

DESCRIPTION

Oxoid P.K.U. Test Agar has been developed from materials that will ensure maximum sensitivity and reliability in its use for screening tests. The specially selected agar gives the medium a perfect transparency which enables the laboratory worker easily to detect even traces of bacterial growth.

The medium is based on the formulation of Demain (1958), modified by Guthrie and Susi (1963).

The Guthrie test is made on filter paper which has been impregnated with several spots of blood (obtained from heel-pricks of 6-day-old infants), dried, and forwarded to the central processing laboratory.

In the laboratory a small disc punched from one of the blood spots on the filter paper is incubated overnight on an assay dish containing spores of *Bacillus subtilis* ATCC6051 and a substance (β-2-thienylalanine) which inhibits growth. A series of control discs impregnated with various concentrations of phenylalanine, which permits growth of *B. subtilis* in the presence of the inhibitor, are also incubated on the dish. Comparison of the halo of growth of *B. subtilis* around the test disc with that around the control discs gives an assessment of the phenylalanine content of the test blood.

The Guthrie test detects and estimates concentrations of phenylalanine down to about 2 mg/100 ml of blood. A level of 6 mg/100 ml, or more, is regarded as a result requiring fuller investigation (M.R.C., 1968).

The disease of phenylketonuria is characterised by an excessive level of phenylalanine in the serum, appearing within a few days of birth and usually resulting, if left untreated, in mental retardation. The disorder is due to a block in the metabolism of phenylalanine to tyrosine, and it is the phenylpyruvic acid which may be detected in the urine that gives rise to the name phenylketonuria.

The publications of Guthrie and Guthrie *et al.* (1961a, 1961b, 1962, 1963, 1965, 1969) should be consulted for details of the test.

REFERENCES

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Medical Research Council. Working Party on Phenylketonuria (1968) 'Present status of different mass screening procedures for phenylketonuria' *B.M J.* **ii**, 7–13.

Plate Count Agar

Tryptone Glucose Yeast Agar

Code—Powder CM325
Tablets CM326

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	5.0
Yeast Extract Powder (Oxoid L21)		2.5
Dextrose		1.0
Agar No. 1 (Oxoid L11)		9.0

pH 7.0 (approx.)

DIRECTIONS

Powder: Suspend 17.5 g in 1 litre of distilled water. Dissolve by bringing to the boil with frequent stirring, mix and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Plate Count Agar is equivalent to the medium recommended by the APHA (1960) for the plate count of micro-organisms in milk and other dairy products. It supersedes Tryptone Glucose Extract Agar for this purpose but the new medium does not require the addition of skim milk.

Basically the APHA method consists of the preparation of pour-plates using diluted samples, and counting colonies after incubation. Incubation is for 48 hours at 32° C or at 35° C for the Standard Plate Count. For the enumeration of micro-organisms with other temperature requirements, plates may also be incubated for 7–10 days at 5–7° C; for 3–5 days at 20° C; for 2–3 days at 45° C; or for 48 hours at 55° C. See the APHA (1960) publication for details.

REFERENCE

American Public Health Association (1960) 'Standard Methods for the Examination of Dairy Products' 11th ed., APHA Inc., New York.

Plate Count Agar (ROLL TUBE)

Code—Powder CM325a
Tablets CM326a

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**5.0**
Yeast Extract Powder (Oxoid L21)		**2.5**
Dextrose		**1.0**
Agar No. 1 (Oxoid L11)		**15.0**

pH 7.0 (approx.)

DIRECTIONS

Add 23.5 g to 1 litre of distilled water and boil to dissolve the medium completely. Distribute in 4.0 or 4.5 ml amounts in Astell tubes and loosely insert the seals. Sterilize by autoclaving for 15 minutes at 121° C. Immediately on removal from the autoclave, press home the seals.

DESCRIPTION

Plate Count Agar (Roll Tube) has been especially reinforced with added agar to enable colony counts on milk and other dairy products to be carried out in Roll Tubes.

TECHNIQUE

Cool the medium in a water bath at 43–45° C. Add 0.5 or 1.0 ml of diluted milk to each bottle and immediately seal and place on the spinning machine. Remove the spun bottles after solidification of the medium and incubate seal downwards for 48 hours at 32° C or 35° C for the standard plate count.

Standard Plate Count Agar (A.P.H.A)

See page 251.

Potassium Lactate 50%

Code SR37

For use with Lysine Medium CM191. Potassium Lactate 50% is available in 1 ml ampoules packed in boxes of ten.

Potassium Tellurite 3.5%

Code SR30

A selectively inhibitory agent which is added to Baird-Parker Medium CM275, Cholera Medium CM237, Hoyle Medium Base CM83 and Mitis Salivarius Agar Base CM157. Potassium Tellurite 3.5% is available in 2 ml ampoules packed in boxes of ten.

Potato Dextrose Agar

Code—Powder CM139
Tablets CM140

FORMULA

Potato Extract	*grams per litre*	**4**
Dextrose		**20**
Agar No. 1 (Oxoid L11)		**15**

pH 5.6 (approx.)

DIRECTIONS

Powder: Suspend 39 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

In order to suppress bacterial growth it is sometimes desirable to acidify the medium to pH 3.5. This can be done by adding 1.0 ml of Tartaric Acid 10% SR36 to each 100 ml of sterilized medium at 50° C. The medium must not be heated after the addition of the acid, this would result in hydrolysis of the agar and destroy its gelling properties.

DESCRIPTION

A suitable medium for the isolation and count of yeasts and moulds in butter etc. (American Public Health Association, 1953) or those occurring on the surface of fresh meats, cured meats and sausage products (American Public Health Association, 1966).

TECHNIQUE

After sterilizing the reconstituted medium adjust the reaction to pH 3.5 by adding 1 ml of Tartaric Acid 10% SR36 to each 100 ml of medium at 50° C. Do not reheat after acidification.

Prepare dilute emulsions or suspensions of the product to be tested, make pour-plates in the usual manner, and incubate for 5 days at 21° C. Count the number of yeast and mould colonies.

If a non-selective medium is required, it is suggested that Potato Dextrose Agar may be used without added acid or, alternatively, one may use a general purpose mycological medium such as Malt Extract Agar CM59.

REFERENCES

American Public Health Association (1953) 'Standard Methods for the Examination of Dairy Products' 10th ed., APHA Inc., New York.
American Public Health Association (1966) 'Recommended Methods for the Microbiological Examination of Foods' 2nd ed., APHA Inc., New York.

Proteolysed Liver

See page 210.

Proteose Peptone

See page 213.

Purple Serum Agar Base

Code CM121

FORMULA

	grams per litre
Peptone (Oxoid L37)	20.0
Lactose	20.0
Sodium chloride	5.0
Phenol red	0.024
Bromo-cresol purple	0.030
Agar No. 3 (Oxoid L13)	15.0

pH 7.6 (approx.)

DIRECTIONS

Suspend 60 g in 1 litre of distilled water. Boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C and add 70 ml of sterile Horse Serum SR35, mix well and pour plates.

DESCRIPTION

This medium, described by Coates (1954), is for the cultivation and differentiation of urinary organisms. Purple Serum Agar is sufficiently nutritious to provide a luxuriant growth of all organisms likely to be encountered in the urinary tract, and also provides a means whereby Gram-negative bacilli may be readily differentiated.

COLONIAL APPEARANCE

After 18 to 24 hours' incubation at 37° C, typical colonial characteristics are as follows:

Escherichia coli—1.5 to 4 mm diameter, translucent to opaque, convex and yellow.

Staphylococcus aureus—1 to 3 mm diameter, opaque, discrete convex colonies. Usually yellow due to lactose fermentation but strains vary in their reactions.

Streptococcus pyogenes—0.5 to 1 mm diameter, translucent, discrete, pale yellow.

Streptococcus faecalis—1 to 2 mm diameter, translucent to opaque, discrete, brilliant yellow surrounded by a marked and constant zone of precipitation in the medium.

Non-lactose fermenting enteric organisms—1 to 3 mm diameter, moist, translucent, convex, lilac blue.

Proteus species—lilac blue, spreading moist growth.

Pseudomonas aeruginosa—mucoid, confluent growth with greenish pigment.

REFERENCE

Coates, L. V. (1954) 'Purple Serum Agar—a simple differential medium for the cultivation of urinary pathogens' *J. Med. Lab. Technol.* **12**(4), 187–190.

Pyruvic Acid Egg Medium

Code PM2A

FORMULA

Egg Fluid	1 litre
Pyruvic acid	3.0 g
Potassium dihydrogen phosphate	11.4 g
Disodium hydrogen phosphate	6.0 g
Magnesium sulphate	0.3 g
Malachite green	0.125 g
Penicillin G	100,000 units

DESCRIPTION

Stonebrink (1958, 1961) demonstrated the value of pyruvic acid in the cultivation of mycobacteria and presented evidence of enhanced growth of *Myco. bovis* and dysgonic strains of *Myco. tuberculosis*.

Marks (1963) extended this work and confirmed the findings of Stonebrink, especially in the presence of reduced malachite green concentration. The 'Oxoid' medium is made to Marks' formula.

Although pyruvic egg medium is superior to glycerol medium, Marks comments that the use of both media is to be recommended. This is because pyruvic acid may discourage the growth of certain anonymous mycobacteria, especially *Myco. kansasii*.

Hughes (1966) confirmed Marks' comment that pyruvic acid medium is superior to glycerol, inasmuch as 5% of his isolated strains grew on pyruvate medium only.

Cruickshank (1965) recommended the use of pyruvic acid medium for the isolation of human strains that are drug-resistant and difficult to grow.

REFERENCES

Marks, J. (1963) 'Pyruvic Acid in the Cultivation of Tubercle Bacilli' *Mon. Bull. Min. Hlth and P.H.L.S.* **22**, 150–152.
Stonebrink, B. (1958) *Acta tuberc. scand.* **35**, 67.
Stonebrink, B. (1961) 'Selected Papers of the Royal Netherlands Tuberculosis Association **2**, 22.
Cruickshank, R. (1965) 'Medical Microbiology', 11th ed., p. 754. Livingstone, Edinburgh.
Hughes, M. H. (1966) *J clin. Path* **19**, 73.

Reinforced Clostridial Agar

RCM Agar
Code CM151

FORMULA

	grams per litre
Yeast Extract (Oxoid L20)	**3.0**
'Lab-Lemco' Powder (Oxoid L29)	**10.0**
Peptone (Oxoid L37)	**10.0**
Dextrose	**5.0**
Soluble starch	**1.0**
Sodium chloride	**5.0**
Sodium acetate	**3.0**
Cysteine hydrochloride	**0.5**
Agar No. 3 (Oxoid L13)	**15.0**

pH 6.8 (approx.)

DIRECTIONS

Suspend 52.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 116° C for 20 minutes.

DESCRIPTION

Reinforced Clostridial Agar is a solid version of Oxoid Reinforced Clostridial Medium CM149, suitable for the cultivation and enumeration of clostridia and other anaerobes, lactobacilli, and many other species of bacteria. Reinforced Clostridial Agar does not differ significantly in performance from the pork-starch-pea agar of Anderson (1951) for the count of anaerobes. It is employed for the estimation of clostridia in food—see below (also Barnes *et al.,* 1963 and Angelotti, 1962). Attenborough & Scarr (1957) employed RCM agar, in conjunction with Oxoid Membrane Filters, for the count of *Clostridium thermosaccharolyticum* in sugar—see page 155.

Reinforced Clostridial Agar is also frequently employed for the investigation of intestinal flora: Perry *et al.* (1955) for investigation of bovine rumen streptococci; Williams Smith & Crabb (1961) used the Oxoid medium with added sodium chloride or blood for counts on human or animal faeces; Barnes & Goldberg (1962) employed the Oxoid medium with added chlortetracycline hydrochloride or sodium azide and ethyl violet, for the examination of poultry faeces; the Oxoid medium was also used by Goldberg *et al.* (1964) for the examination of poultry faecal samples. Williams Smith (1961) employed Oxoid Reinforced Clostridial Agar with added blood for the 'total' and *Lactobacillus* count of human and animal faeces; with added blood and neomycin for determination of *Bacteroides,* anaerobic Gram-negative cocci, 'total' streptococci, sporeformers, yeasts, and 'actino' types.

Sneath (1962) used Oxoid RCM Agar and other media for the anaerobic count of micro-organisms from soil samples up to more than 300 years old. Gregory *et al.* (1963) also employed the Oxoid medium for the estimation of anaerobes in moulding hay.

TECHNIQUE

Barnes & Ingram (1956), Ingram & Barnes (1956) described the use of RCM agar for the total viable count of clostridia employing their black glass rod technique. In this method the diluted sample is added to plugged test tubes containing about 9 ml of RCM Agar held at 48° C. The tubes are quickly rotated to mix the contents, and a sterile black glass rod inserted into each tube before the agar sets. They are then sealed with about 1.5 cm of RCM Agar containing 1/20,000 methylene blue. Growth is very rapid in this medium and it is necessary to count the colonies before gas production disrupts the agar. The authors suggest that the tubes should be incubated overnight at 25° C and then at 37° C for several hours. The colonies are clearly visible against the black background (Ingram & Barnes, 1956).

Oxoid RCM Agar may also be used for enumerating anaerobes using the Miller-Prickett technique (Miller *et al.,* 1939). The Miller-Prickett tube is a flattened test tube 15 × 2.5 × 1.3 cm now obtainable from the Astell Laboratory Service Co. Ltd., 172 Brownhill Road, London, S.E.6. Mossel *et al.* (1956) although working with other media, suggested the following procedure which may be used with RCM Agar:

1. Transfer, in triplicate, 1 ml of serial decimal dilutions of the food under investigation into sterilized, plugged Miller-Prickett tubes. Cool the freshly prepared medium to approximately 50° C and, without shaking, add about 15 ml to each tube.

2. Seal immediately with melted sterile paraffin, and allow to set in a water bath at about 15° C.

3. Incubate for 1 to 10 days at a temperature between 30° C and 55° C, depending on the type of clostridia expected.

4. Run at least one blank to detect contamination occurring during the procedure.

5. Count colonies.

REFERENCES

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Barnes, Ella M., and Goldberg, H. S. (1962) 'The Isolation of Anaerobic Gram-Negative Bacteria from Poultry Reared with and without Antibiotic Supplements' *J. appl. Bact.* **25**(1), 94–106.

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Ingram, M. and Barnes, Ella M. (1956) 'A Simple Modification of the Deep Shake Tube for Counting Anaerobic Bacteria' *Lab. Practice* **5**(4), 145.

Miller, N. J., Garrett, O. W. and Prickett, P. S. (1939) 'Anaerobic Technique—A Modified Deep Agar Shake' *Food Res.* **4**(5), 447–451.

Mossel, D. A. A., De Bruin, A. S., Diepen, H. M. J. van Vendrig, C. M. A. and Zoutwelle, G. (1956) 'The Enumeration of Anaerobic Bacteria, and of *Clostridium* Species in particular, in Foods' *J. appl. Bact.* **19**(1), 142–154.

Perry, K. D., Wilson, M. K., Newland, L. G. M. and Briggs, C. A. E. (1955) 'The Normal Flora of the Bovine Rumen. III Quantitative and Qualitative Studies of Rumen Streptococci' *J. appl. Bact.* **18**(3), 436–442.
Sneath, P. H. A. (1962) 'Longevity of Micro-organisms' *Nature* **195**(4842), 643–646.
Williams Smith, H. (1961) 'The Development of the Bacterial Flora of the Faeces of Animals and Man: the changes that occur during ageing' *J. appl. Bact.* **24**(3), 235–241.
Williams Smith, H. and Crabb, W. E. (1961) 'The Faecal Bacterial Flora of Animals and Man: its development in the young' *J. Path. Bact.* **82**(1), 53–66.

Reinforced Clostridial Medium

R.C.M.

Code CM149

FORMULA

	grams per litre
Yeast Extract (Oxoid L20)	**3.0**
'Lab-Lemco' Powder (Oxoid L29)	**10.0**
Peptone (Oxoid L37)	**10.0**
Soluble starch	**1.0**
Dextrose	**5.0**
Cysteine hydrochloride	**0.5**
Sodium chloride	**5.0**
Sodium acetate	**3.0**
Agar	**0.5**

pH 6.8 (approx.)

DIRECTIONS

Suspend 38 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115° C for 20 minutes.

DESCRIPTION

A semi-solid medium for the enumeration and cultivation of anaerobes. Recommended for the isolation and cultivation of anaerobic organisms occurring in a variety of habitats, including food and pathological specimens.

Reinforced Clostridial Medium (R.C.M.) was designed by Hirsch & Grinstead (1954) for the cultivation and enumeration of clostridia. They showed that the medium was more fertile and enabled growth to be initiated from small inocula more readily than five other media tested. In a further comparison, the highest viable count obtainable was the criterion used, and again, R.C.M. proved superior. Compared with the spleen infusion medium of Mundt & Jones (1952), R.C.M. gave somewhat higher counts (Gibbs & Hirsch, 1956).

In their quantitative studies Wilson & Briggs (1955) reported that the medium which gave the best results on normal bovine rumen flora was R.C.M. This medium gave a higher viable count than nine other media tested including a broth specially designed for rumen bacteria. R.C.M. has been advocated as a diluent for rumen bacteria by Wilson & Briggs (1954) and more recently Barnes & Ingram (1956) used this medium for diluting an inoculum of vegetative cells of *Clostridium perfringens* (*Cl. welchii*). Perry *et al.* (1955) employed R.C.M. and R.C.M. buffered with sodium bicarbonate and carbon dioxide, in their dilution counts and in the purification of isolates of *Streptococcus bovis*. Oxoid R.C.M. is the outcome of considerable development research and although it contains the originally recommended concentration of agar, it remains free from the light but troublesome precipitate mentioned by Gibbs & Hirsch (1956). It is recommended that R.C.M. be distributed into 1 oz. screw-capped bottles, which do not require petroleum jelly or other anaerobic seals.

Barnes & Goldberg (1962) and Goldberg *et al.* (1964) employed Oxoid Reinforced Clostridial Medium with additions for the isolation of *Bacteroides*-like organisms from poultry caeca.

A solidified version of this medium is available—see Reinforced Clostridial Agar CM151, page 228.

REFERENCES

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Wilson, Marion K. and Briggs, C. A. E. (1954) 'The Normal Flora of the Bovine Rumen. Bacteriological Methods for Quantitative Studies' *Vet. Rec.* **66**, 187–188.
Wilson, Marion K. and Briggs, C. A. E. (1955) 'The Normal Flora of the Bovine Rumen. II Quantitative Bacteriological Studies' *J. appl. Bact.* **18**(2), 294–306.

Resuscitation Membrane Broth Tablets

Code MM20

FORMULA

	grams per litre
Peptone (Oxoid L37)	**40**
Yeast Extract (Oxoid L20)	**6**
Lactose	**30**

pH 7.4 (approx.)

DIRECTIONS

Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 121° C for 15 minutes.

Corresponding to the MWB formulation, this medium is used to resuscitate coliform organisms prior to cultivation on the differential medium MM6a. During isolation, its is also advantageous to resuscitate other Enterobacteriaceae on MM20 before cultivation on selective media.

For further details of membrane filtration techniques, see the separate booklet entitled 'Membrane Filtration'.

Ringer Solution
¼ Strength Ringer Solution Tablets

Code BR52

FORMULA

	grams per litre
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride	0.12
Sodium bicarbonate	0.05

pH 7.0

One tablet makes 500 ml of ¼-strength Ringer solution

DIRECTIONS

To prepare quarter-strength Ringer Solution, dissolve 1 tablet in 500 ml of distilled water. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

These tablets are prepared according to the formula in the Dept. of Health & Social Security publication Memo. 139/Foods 1937. They dissolve readily in water to give a solution which does not precipitate when sterilized by autoclaving. The sterile solution is used as an isotonic diluent for both bacterial cells and bacteriological specimens, or as a rinse during the bacteriological examination of plant and apparatus.

TECHNIQUE

Oxoid quarter-strength Ringer Solution may be used for: the decimal dilution of milk and dairy product samples, e.g. prior to inoculation into MacConkey Broth for the presumptive coliform test; the maceration and suspension of solid food and other specimens before bacteriological examination; the preparation of suspensions and serial dilutions from pure cultures of bacteria; the examination of dairy plant and apparatus by the swab or rinse method (Davis, 1956).

See also Thiosulphate Ringer Tablets BR48, page 207, and 'Calgon' Ringer Tablets BR49, page 206.

REFERENCES

Davis, J. G. (1956) 'Laboratory Control of Dairy Plant', Dairy Industries Ltd., London.
Dept. of Health & Social Security (1937) 'Bacteriological Tests for Graded Milk' Memo. 139/Foods.

'Calgon' Ringer Tablets

Code BR49

FORMULA

	grams per litre
Sodium chloride	2.25
Potassium chloride	0.105
Calcium chloride	0.12
Sodium bicarbonate	0.05
Sodium hexametaphosphate ('Calgon')	10.0

pH 7.0

ONE TABLET MAKES 10 ml OF ¼-STRENGTH RINGER CONTAINING 1% OF 'CALGON'. (*Sodium hexametaphosphate.*)

DIRECTIONS

To prepare a solution containing 1% sodium hexametaphosphate in quarter-strength Ringer Solution, dissolve 1 tablet in 10 ml of distilled water. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Higgins (1950a) showed that bacteriological swabs prepared from calcium alginate wool ('Calgitex'*) will dissolve completely in 'Calgon' Ringer Solution, thus releasing all the organisms taken up on the swab and giving a more accurate quantitative recovery. She concluded that the use of this material in place of cotton-wool for the preparation of swabs, seemed justified in quantitative work since the recovery of organisms was much greater.

This principle was successfully applied to the bacteriological examination of tableware (Higgins & Hobbs, 1950) and also of crockery and kitchen utensils (Higgins, 1950b).

Trimarchi (1959) found that calcium alginate swabs were superior to raw cotton swabs for the bacteriological examination of eating utensils.

TECHNIQUE

Prepare swabs, using approximately 25–50 milligrams of alginate wool to each wooden applicator. Sterilize, in plugged tubes, by autoclaving at 121° C for 15 minutes. After swabbing, aseptically break off the swab end and drop into a screw-top bottle containing 10 ml of sterile 'Calgon' Ringer Solution. Close the bottle and shake vigorously for ½ to 1 minute to dissolve the alginate wool completely. The suspension may then be examined quantitatively or qualitatively in the usual manner.

REFERENCES

Higgins, Marcella (1950a) 'A Comparison of the Recovery Rate of Organisms from Cotton-Wool and Calcium Alginate Wool Swabs' *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv.* 9, 50–51.
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Higgins, Marcella and Hobbs, Betty C. (1950) 'Kitchen Hygiene: The Effectiveness of Current Procedure in Cleansing Tableware' Ibid., 38–49.
Trimarchi, G. (1959) 'The Bacteriological Control of Food Utensils in Public Service. (1) Methods for the Determination of the Bacterial Content' *Igiene Moderna* 52(1/2), 95–111.

*A purified bacteriological grade prepared by Medical Alginates Ltd., Wadsworth Road, Perivale, Middx.

Thiosulphate Ringer Tablets

Code BR48

FORMULA

Sodium chloride	*grams per litre*	**2.15**
Potassium chloride		**0.075**
Calcium chloride		**0.12**
Sodium thiosulphate		**0.5**

pH 6.6

One tablet makes 500 ml of ¼-strength Ringer containing 0.05% of Sodium thiosulphate

DIRECTIONS

To prepare quarter-strength Ringer Solution containing thiosulphate, dissolve 1 tablet in 500 ml distilled water. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Oxoid Thiosulphate Ringer Tablets are formulated to provide, after the addition of distilled water, a Ringer Solution (British Pharmacopoeia, 1953) containing 0.05% of sodium thiosulphate hydrate. Use of the B.P. Ringer basis instead of the Ministry of Health Ringer ensures chemical stability without altering the physiological properties of the diluent. The purpose of the sodium thiosulphate is to neutralize residual chlorine present in bacteriological samples and rinses as a result of the previous use of gaseous chlorine or chlorine-containing disinfectants. The thiosulphate immediately terminates the bactericidal action of the chlorine in the sample—so that subsequent bacteriological examination more accurately indicates the pollution of the sample at the time of collection. Sterile Thiosulphate Ringer Solution is consequently employed for rinse water samples taken for sanitary tests (from equipment and pipe lines, etc.) where a chlorine containing cleansing fluid has been used. Davis (1956) used a Ringer Solution with added sodium thiosulphate, as a rinse after the use of sodium hypochlorite. Egdell *et al.* (1958) employed a Thiosulphate Ringer Solution in their bacteriological studies of immersion cleaned milking equipment.

TECHNIQUE

100 ml of prepared solution will completely neutralize 7 mg of chlorine. Oxoid Thiosulphate Ringer Solution may be used for assessing the cleanliness and sterility of dairy plant, after hypochlorite solution has been used for disinfection purposes (see Davis, 1956). The methods described by Davis rely on swabbing or rinsing of apparatus or milk bottles. In reference to the swab method, see 'Calgon' Ringer Tablets.

REFERENCES

British Pharmacopoeia (1953), p. 501.

Davis, J. G. (1956) 'Laboratory Control of Dairy Plant' Dairy Industries Ltd., London.

Egdell, J. W., Lomax, K. L., Adams, R. P. and Aitken, M. J. (1958) 'Bacteriological Studies of Immersion Cleaning of Milking Equipment on Commercial Farms' *J. appl. Bact.* 21(1) 109–117.

Rogosa Agar

Code PM221

FORMULA

Tryptone (Oxoid L42)	*grams*	**10.0**
Yeast Extract (Oxoid L20)		**5.0**
Dextrose		**20.0**
'Tween' 80		**1.0**
Potassium dihydrogen phosphate		**6.0**
Ammonium citrate		**2.0**
Sodium acetate		**25.0**
Glacial acetic acid		**1.32 ml**
$MgSO_4.7H_2O$		**0.575**
$MnSO_4.2H_2O$		**0.12**
$FeSO_4.7H_2O$		**0.034**
Agar No. 3 (Oxoid L13)		**20.0**

and distilled water to 1 litre

pH 5.4 (approx.)

DIRECTIONS

Rogosa Agar, a modification of the medium described by Rogosa *et al.* (1951), is a selective medium for the isolation and enumeration of lactobacilli. The medium has given excellent results when used in quantitative and qualitative studies of lactobacilli in faeces, saliva and mouth rinses, and in dairy products.

TECHNIQUE

For the isolation of lactobacilli, Sharpe (1960) recommends that Rogosa agar plates should be incubated for 3 days at 37° C or for 5 days at 30° C. It is preferable to incubate in an atmosphere containing 95% of hydrogen and 5% of carbon dioxide, this prevents evaporation, provides the microaerophilic conditions favoured by lactobacilli, and the carbon dioxide has a stimulating effect on their growth. If a suitable container is not available, overlay the inoculated plate with a second layer of Rogosa Agar, before incubation.

REFERENCES

Rogosa, M., Mitchell, J. A. and Wiseman, R. F. (1951) 'A Selective Medium for the Isolation of Oral and Fecal Lactobacilli' *J. Bact.* 62(1), 132–133.

Sharpe, M. Elizabeth (1960) 'Selective Media for the Isolation and Enumeration of Lactobacilli' *Lab. Practice* 9(4), 223–227.

Sabouraud Dextrose Agar

Code—Powder CM41
Tablets CM42

FORMULA

Mycological Peptone (Oxoid L40)	*grams per litre*	**10**
Dextrose		**40**
Agar No. 1 (Oxoid L11)		**15**

pH 5.6 (approx.)

DIRECTIONS

Powder: Suspend 65 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This modification of Sabouraud agar (Carlier, 1948) is suitable for the cultivation and differentiation of fungi.

Carlier showed that the medium gives reliable results with *Microsporum audouini, M. canis, Trichophyton mentagrophytes, T. flavum, T. rubrum* and *Candida albicans.* Sabouraud Dextrose Agar may be used in place of the Standard American medium of Hodges (1928). The fungi maintain their typical cultural appearance and thus may be readily identified according to the standard macroscopic characters described by Sabouraud (1910).

TECHNIQUE

The medium is often used with antibiotics for the isolation of pathogenic fungi, from material containing large numbers of other fungi or bacteria. Georg *et al.* (1954) aseptically added 0.5 gram cycloheximide, 20,000 units penicillin and 40,000 units streptomycin to each litre of autoclaved, cooled medium. *Cryptococcus neoformans, Aspergillus fumigatus* and *Allescheria boydii* are sensitive to cycloheximide; *Actinomyces bovis* and *Nocardia asteroides* are sensitive to penicillin and streptomycin.

Alternatively, one may add 0.4 gram chloramphenicol and 0.5 gram cycloheximide to each litre of reconstituted medium before autoclaving (Ajello, 1957). The same micro-organisms are sensitive to this new combination—see above.

Williams Smith & Jones (1963) employed Oxoid Sabouraud Dextrose Agar, containing 20,000 units penicillin and 0.04 gram neomycin per litre, for the count of yeasts in the alimentary tract of the pig.

Oxoid Sabouraud Dextrose Agar may also be used as the basis of a Pagano-Levin medium (Pagano *et al.*, 1957–58) for the isolation of *Candida albicans.* 0.1 gram of triphenyltetrazolium chloride (as a filter sterilized solution) is added to each litre of autoclaved molten medium cooled to 55° C. The medium is usually made inhibitory to most non-pathogenic fungi and bacteria by the addition of antibiotics as above. After incubation for 3 days at 25° C, *Candida albicans* colonies are unpigmented or pale pink whilst other *Candida* species and other fungi form deeper pink or red colonies. The Pagano-Levin technique has been investigated by Kutscher *et al.* (1959), Sinski (1960), Ridley (1960) and several others; the test is adequate for screening purposes but other diagnostic criteria should also be utilized for the identification of *Candida albicans.*

REFERENCES

Ajello, Libero (1957) 'Cultural Methods for Human Pathogenic Fungi' *J. Chron. Dis.* **5**(5), 545–551.

Carlier, Gwendoline I. M. (1948) *Brit. J. Derm. Syph.* **60,** 61–63.

Georg, Lucille K., Ajello, L. and Papageorge, Calomira (1954) *J. Lab. Clin. Med.* **44**(3), 422–428.

Hodges, R. S. (1928) *Arch. Derm. Syph.*, New York, **18,** 852.

Kutscher, A. H., Seguin, L., Zegarelli, E. V., Rankow, R. M., Mercadante, J. and Piro, J. D. (1959a) 'Growth Characteristics of *Candida albicans* on Pagano-Levin Culture Medium' *J Invest Derm.* **33,** 41–47.

Kutscher, A. H., Seguin, L., Zegarelli, E. V., Rankow, R. M., Campbell, J. B. and Mercadante, J. (1959b) 'Pagano-Levin Culture Medium for Differentiation of *Candida albicans*' American Type Culture Collection Studies. 1. *Antibiotics and Chemotherapy* **9**(11), 649–659.

Pagano, J., Levin, J. G. and Trejo, W. (1957–58) 'Diagnostic Medium for Differentiation of Species of *Candida*' Antibiotics Annual 1957–58, 137–143.

Ridley, M. F. (1960) 'A Comparison of Methods for Identification of *Candida albicans* *Australian J. Dermat.* 5(4), 209–213.

Sabouraud, R. (1910) 'Les Teignes', Masson, Paris.

Sinski, J. T. (1960) 'The Effectiveness of Pagano-Levin Medium for the Detection and Identification of *Candida albicans* in Clinical Specimens' *J. invest. Dermat.* **35**(3), 131–133.

Williams Smith, H. and Jones, J. E. T. (1963) 'Observations on the Alimentary Tract and its Bacterial Flora in Healthy and Diseased Pigs' *J. Path. Bact.* **86**(2), 387–412.

Sabouraud Liquid Medium

Code—Powder CM147
Tablets CM148

FORMULA

Pancreatic digest of casein (Oxoid L42) *grams per litre*	**5**
Peptic digest of fresh meat (Oxoid L49)	**5**
Dextrose	**20**

pH 5.7 (approx.)

DIRECTIONS

Powder: Add 30 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Sabouraud Liquid Medium is a mycological sterility test medium conforming with the medium described in the United States Pharmacopeia (1965) for the determination of the fungistatic activity of pharmaceutical products in order to avoid false sterility tests. The medium may also be recommended for the cultivation of moulds, yeasts, and acidophilic bacteria.

TECHNIQUE

The U.S.P. recommends that the fungistatic activity of pharmaceutical products be determined as follows:

(i) Test Culture. A 1 in 1,000 dilution of a 24 to 28 hour culture of *Candida albicans* (*Monilia albicans*) in Fluid Sabouraud Medium.

(ii) Tests. Add specified amounts of the product to be tested to volumes of Fluid Sabouraud Medium and inoculate with the Test Culture.

(iii) Controls. Inoculate tubes of Fluid Sabouraud Medium only, with the Test Culture.

(iv) Incubate at 22° C to 25° C for at least 10 days.

(v) If growth in the Test series is comparable to that in the Control tubes, then the product is not fungistatic—therefore use the amount of product and medium specified for all routine sterility tests on the product.

If the product is fungistatic when tested as above—add a suitable sterile inactivating reagent, or, use a larger ratio of medium to product in order 'to determine the ratio of product to medium in which growth of the test organism is not affected.

REFERENCE

Pharmacopeia of the United States': Seventeenth Revision (1965), p. 840.

Sabouraud Maltose Agar

Code—Powder CM41a
Tablets CM42a

FORMULA

Mycological Peptone (Oxoid L40)	*grams per litre*	**10**
Maltose		**40**
Agar No. 1 (Oxoid L11)		**15**

pH 5.6 (approx.)

This medium differs from Sabouraud Dextrose Agar, only in the carbohydrate incorporated. Sabouraud Maltose Agar may be used, with or without antibiotics, where a maltose medium is preferred.

Sabouraud Maltose Agar may be modified to form a selective indicator medium for the isolation of *Candida albicans* by the addition of Tergitol-7, bromocresol purple, potassium tellurite and triphenyltetrazolium chloride (Chapman, 1952). See also page 209.

REFERENCE

Chapman, G. H. (1952) 'The Isolation and Differentiation of *Monilia* and Other Fungi' *Trans. New York Acad. Sci.*, Series II **14**(6), 254.

Salicin 2.5%

SR10

Filter sterilized solution, available in 5 ml ampoules, in boxes of ten.

Saline Tablets (FOR LABORATORY USE ONLY)

Code BR53

Dissolve 1 tablet in 500 ml of distilled water in order to obtain 0.85% ('normal', physiological, or isotonic) saline.

Salmonella Shigella Agar

See page 251.

Salt Meat Broth

Code CM94

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**10**
'Lab-Lemco' Powder (Oxoid L29)		**10**
Neutral Ox-heart tissue		**30**
Sodium chloride		**100**

pH 7.6 (approx.)

DIRECTIONS

Add 2 tablets to 10 ml of distilled water in a ⅝ in. diameter test tube and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Salt Meat Broth is an enrichment medium for the isolation of staphylococci from grossly contaminated specimens such as faeces, particularly during the investigation of staphylococcal food poisoning. Maitland & Martyn (1948) and Fairbrother & Southall (1950) showed that a salt meat medium will detect small numbers of staphylococci when mixed with large numbers of other bacteria.

The medium is also an excellent substrate for the cultivation of some of the halophilic micrococci associated with hides and raw salt supplies. It should be noted that staphylococci growing on this medium cannot be directly tested for coagulase production—they should first be subcultured on a medium which contains less salt, Blood Agar Base CM55 is recommended for this purpose.

TECHNIQUE

For the isolation of *Staphylococcus aureus* from pathological specimens of food, emulsify the specimen in Peptone Water CM9, and inoculate a tube of Salt Meat Broth. After 24 to 48 hours' incubation at 37° C, discrete colonies may be obtained by plating out a small portion of the liquid culture on Mannitol Salt Agar CM85 or Staphylococcus Medium No. 110 (Code CM145).

REFERENCES

Fairbrother, R. W. and Southall, J. E. (1950) 'The isolation of *Staphylococcus pyogenes* from faeces' *Mon. Bull. Min. Hlth. Pub. Hlth. Lab. Serv.* **9**, 170–172.
Maitland, H. B. and Martyn, G. (1948) 'A Selective Medium for Isolating Staphylococcus Based on the Differential Inhibiting Effect of Increased Concentrations of Sodium Chloride' *J. Path. Bact.* **60**, 553.

Schaedler Medium

Code CM437

FORMULA

Tryptone Soya Broth (Oxoid CM 129)	*grams per litre*	**10.0**
Special Peptone (Oxoid L72)		**5.0**
Yeast Extract Powder (Oxoid L21)		**5.0**
Dextrose		**5.0**
Cysteine HC1		**0.4**
Haemin		**0.01**
Tris Buffer		**0.75**
Agar No. 3 (Oxoid L13)		**13.5**

pH 7.6 (approx)

DIRECTIONS

Suspend 40 g in 1 litre of distilled water and boil to dissolve the medium completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring.

DESCRIPTION

Schaedler Medium was developed for the selection of anaerobic from aerobic micro-organisms (Schaedler, Dubos & Castello, 1965) and the medium can be used to create selective conditions under which the required delicate and more nutritionally exacting micro-organisms of the intestinal tract would develop, despite the presence of antagonistic organisms. Normally such fastidious micro-organisms would be swamped by the growth of enterococci, coliform bacilli and other gram-negative bacilli.

Schaedler described the use of the base medium with selective agents (shown overleaf) for the isolation and enumeration of Lactobacilli, Streptococci, Clostridia, Bacteroides and Flavobacterium species from faecal samples and various organs of the digestive tract.

Although thioglycollate is widely used in anaerobic media, to lower the redox potential in order to favour growth of anaerobic organisms, some workers have reported it to be inhibitory to some anaerobes (Hibbert & Spencer 1970, Mossel, Beerens, Tahon-Castel, Baron & Potpeel, 1965).

Schaedler Medium contains cysteine hydrochloride and glucose, as reducing substances, with the advantage that cysteine inhibits the growth of *Escherichia coli*. Kari, Nagy, Kovacs & Hernadi (1971) have reported the inhibitory effect of cysteine on several enzymatic reactions of Esch. coli in vitro.

Schaedler Medium has been shown to be a suitable alternative to blood agar for the enumeration of clostridia (de Waart & Pouw, 1970) and has been used for the examination of food, waste products and ditch water (de Waart, 1973). These authors showed the necessity for strict anaerobic conditions for the successful recovery of obligate anaerobes when using this medium without the addition of blood.

Investigations at Oxoid have shown that Schaedler Medium gave similar results in recovery and colonial appearance to Oxoid Blood Agar Base No. 2 when tested with the same organisms.

TECHNIQUE

The sample suspension is diluted as necessary in order to obtain separated and countable colonies. A calibrated loopful is then spread on the surface of a previously dried Schaedler Medium plate. Conditions of incubation will vary according to the type of culture under test. Pure cultures may grow on the base medium and this is also used for general aerobic and anaerobic counts.

In the enumeration of *Streptococcus faecalis* (aerobic and facultatively anaerobic) as an indicator organism in dehydrated or frozen foods and water; and for the detection of *Clostridium welchii;* the medium can be used as follows:—

Food sample (e.g. pre-cooked frozen food) suspensions are plated out by the surface spread technique and an aerobic viable count may be carried out at 25° C and 37° C. For pre-cooked meat products, an anaerobic viable count and a selective plate examination for C1. welchii should also be performed.

ADDITION OF SELECTIVE AGENTS

To 1,000 ml of base medium, the following selective agents may be added.

(a) Medium for anaerobic lactobacilli and anaerobic streptococci.

NaC1	10 g
Neomycin	0.002 g

Incubate anaerobically at 37° C

(b) Medium for Bacteroides and Clostridia

Placenta powder	2 g (Nutritional Biochemicals Corp., Cleveland)
Neomycin	0.002 g

Incubate anaerobically at 37° C

(c) Medium for Flavobacterium

7 ml of 0.5% tyrothricin in ethanol.

Incubate aerobically at 37° C

REFERENCES

Schaedler, R. W., Dubos, R. and Castello, R. (1965) 'The development of the bacterial flora in the gastrointestinal tract of mice.' *J. exp. Med.*, **122**, 59–66.

Kari, C., Nagy, Z., Kovacs, P. and Hernadi, F. (1971) 'Mechanism of growth inhibiting effect of cysteine on E. coli.' *J. gen. Micro.*, **68**, 349–356.

Hibbert, H. R. and Spencer, R. (1970) 'An investigation of the inhibitory properties of sodium thioglycollate in medium for the recovery of clostridial spores.' *J. Hyg. Camb.* **68**, 131–135.

Mossel, D. A. A., Beerens, H., Tahon-Castel, Baron, G., and Potspeel, B. (1965). 'A study of media used in food microbiology for the enumeration of spores of anaerobic bacteria.' *Ann. Inst. Pasteur de Lille*, **16**, 147–156.

de Waart, J. and Pouw, H. (1970) 'Studies on the suitability of blood-free media for the enumeration of clostridia.' *Zbl. Bakt.* I. Abt. Orig., **214**, 551–552.

de Waart, J. (1973) Personal Communication.

Selenite Broth

Code CM39

This medium has been withdrawn as the Sodium biselenite in the medium may constitute a health hazard. A leaflet explaining Oxoid's reasons for this decision is available on request.

See **SELENITE BROTH BASE CM395/Sodium Biselenite L.121.**

Selenite Broth Base

(Mannitol Selenite Broth Base, see page 181)

Code—Powder CM395
Tablets CM396
Sodium Biselenite L121

FORMULA

Bacteriological Peptone (Oxoid L34)	*grams per litre*	**5.0**
Lactose		**4.0**
Sodium phosphate		**10.0**

DIRECTIONS

Powder: Add 19 grams to 1 litre of distilled water to which 4 grams of Sodium Biselenite (Oxoid L121) has been added.

Warm to dissolve, mix well and fill out into containers to a depth of 5 cm. Sterilize in a boiling water bath, or in free flowing steam, for 10 minutes.

DO NOT AUTOCLAVE.

Tablets: Dissolve 1 tablet in 10 ml of a solution containing 0.4 grams of sodium biselenite (Oxoid L121) in 100 ml distilled water and sterilize in a boiling water bath or by steaming for 10 minutes. DO NOT AUTOCLAVE.

To minimise any possible risk of teratogenicity to laboratory workers, the sodium biselenite must be added to this medium separately.

DESCRIPTION

Selenite Broth was developed as an enrichment medium for the isolation of *Salmonella typhosa* and other salmonellae from faeces, urine, or other pathological material. This medium is a modification of the original Leifson (1936) Selenite F Broth.

TECHNIQUE

For routine purposes Selenite Broth cultures should be incubated at 37° C for 18 to 24 hours and then subcultured on Bismuth Sulphite Agar (CM201) and Desoxycholate Citrate Agar (CM35 or CM227). The development of *Escherichia coli* and *Proteus* species is not indefinitely retarded in selenite media. Where the initial proportion of these organisms is high, it is often advantageous to subculture on to the solid media after 6 hours as well as after 18 hours.

If a high proportion of debris is present in the sample of material being examined, the selective powers of the selenite may be nullified. This is well established in the examination of faeces and egg powder. It is common practice to emulsify the specimen in sterile saline, allow the gross particles to settle, and inoculate the medium with the supernatant. An alternative method is as follows: add 2 to 3 grams ofsolid specimen to 15 ml of saline in a wide-necked 1 oz. bottle, emulsify, separate the debris by slowly pressing a plug of cotton-wool down through the suspension. Withdraw approximately 1 ml of the supernatant and inoculate 10 ml of Selenite Broth.

Harvey & Scott Thomson (1953) showed that incubation of the selenite broth at 43° C facilitated the isolation of *Salmonella paratyphi B* from faeces. They recommended the use of this principle for the examination of sewage and river water containing large numbers of other bacteria that preferred a lower temperature for growth. The authors also suggested that the procedure was of value for all salmonellae except *Salmonella typhosa*. For urines, the broth should be made double strength and inoculated with its own volume of the specimen.

REFERENCES

Harvey, R. W. S. and Scott Thomson (1953) 'Optimum Temperature of Incubation for Isolation of Salmonellae' *Mon. Bull. Min. Hlth. Pub. Hlth. Lab. Serv.* **12,** 149–150.

Leifson, E. (1936) 'A new Selenite Enrichment Media for the Isolation of Typhoid and Paratyphoid (*Salmonella*) Bacilli' *Am. J. Hyg.* **24**(2), 423–432.

Sensitivity Discs

OXOID Sensitivity Discs are available with a wide range of antibiotic and chemotherapeutic agents in various levels.

For full details of technique and the range available, please ask for the separate 'OXOID' leaflet.

Sensitivity Testing Media

Oxoid manufactures a wide range of products for use in the preparation of Sensitivity Testing Media. These products are listed below and full details of each can be found in this manual alphabetically:

Diagnostic Sensitivity Test Agar-CM 261
Mueller Hinton Agar-CM 337
Mueller Hinton Broth-CM 405
'Iso-Sensitest Agar'-CM-471
'Iso-Sensitest Broth'-CM-473
Blood Products (Horse Blood Laked-SR 48)

Shapton Medium

Code CM270

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**5.0**
Tryptone (Oxoid L42)		**2.5**
'Lab-Lemco' Powder (Oxoid L29)		**3.0**
Yeast Extract (Oxoid L20)		**1.0**
Dextrose		**1.0**
Bromo-cresol purple		**0.025**
Agar No. 3 (Oxoid L13)		**15.0**

pH 7.4 (approx.)

DIRECTIONS

Add 1 tablet to 15 ml or distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This medium, developed for use in the standard thermophilic spore count technique by Shapton & Hindes (1963), is designed for the enumeration of organisms of the *Bacillus stearothermophilus* group, which cause flat-sour spoilage in those canned foods with a pH above 4.5. Although the medium itself is non-selective, the technique devised by Shapton and Hindes permits the selective isolation of flat-sour thermophiles, as large distinctive colonies on the medium.

TECHNIQUE

It is important to read the original paper for exact details of technique but the following is an outline.

The sample, suspended in Ringer Solution is pipetted into molten Shapton Medium. The temperature is raised to 100° C (steam) and maintained for 20 minutes, then raised to 108.4° C (5 p.s.i.) for 10 minutes. After cooling to 50° C it is mixed and poured into Petri Dishes. It is incubated for 48 hours at 55° C and the number of colonies counted.

REFERENCE

Shapton, D. A. and Hindes, W. R. (1963) 'The Standardisation of a Spore Count Technique, Chemistry and Industry', February 9th, pp. 230–234.

Sheep Blood, Defibrinated

(NO PRESERVATIVE)

Code SR51

A product for inclusion in bacteriological culture media, see page 65.

Simmons Citrate Agar

Code—Powder CM155
Tablets CM156

FORMULA

	grams per litre
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromo-thymol blue	0.08
Agar No. 3 (Oxoid L13)	15.0

pH 7.0 (approx.)

DIRECTIONS

Powder: Suspend 23 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Simmons Citrate Agar is recommended (Ewing & Edwards, 1960) for the differentiation of the family Enterobacteriaceae based on whether or not citrate is utilized as the sole source of carbon.

The medium is virtually a solidified form of Koser citrate medium which, in its original form, suffered from the disadvantage that false appearance of growth occurred when large inocula were employed. The addition of bromo-thymol blue indicator to the medium was a distinct improvement. See Koser Citrate Medium CM65, page 150.

TECHNIQUE

The medium may be used either as slopes in test tubes or as a plate medium in Petri dishes. In both cases the surface of the medium is lightly inoculated by streaking and, where slopes are used, the butt of medium is inoculated by stabbing. Incubation for 48 hours at 37° C is recommended.

Positive growth (i.e. citrate utilization) produces an alkaline reaction and changes the colour of the medium from green to bright blue, whilst in a negative test (i.e. no citrate utilization) the colour of the medium remains unchanged.

Faecal coli (*Escherichia coli*) do not grow on the medium whereas most other coliform organisms do. Members of the *Klebsiella-Aerobacter-Serratia Salmonella-Arizona-Citrobacter* and some members of the *Proteus-Providence* divisions all utilize citrate. *Proteus morganii* and the *Escherichia coli* serotypes from epidemic infantile enteritis do not (Kauffmann, 1954).

Simmons Citrate Agar may be employed to differentiate *Salmonella schottmuelleri*, *S. enteritidis* and *S. typhimurium* from *S. typhosa*, *S. paratyphi* and *Shigella*. The latter group does not grow on the medium whereas members of the former group utilize citrate and are able to produce the characteristic blue coloration.

REFERENCES

Ewing, W. H. and Edwards, P. R. (1960) *Internat'l. Bull. Bact. Nomen. and Taxon.* 10(1), 1–12.
Kauffmann, F. (1954) '*Enterobacteriaceae*', 2nd ed., Munksgaard, Copenhagen.

SIM Medium

Code CM435

FORMULA

	grams per litre
Tryptone (Oxoid L42)	20.0
Peptone P (Oxoid L49)	6.1
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Agar No. 1 (Oxoid L11)	3.5

pH 7.3 (approx)

DIRECTIONS

Suspend 30 g in 1 litre of distilled water and boil to dissolve the medium completely. Dispense into final containers and sterilize by autoclaving for 15 minutes at 121° C.

DESCRIPTION

A motility-indole medium has been found to be helpful in the identification of the Enterobacteriaceae; e.g. in the differentiation of Klebsiella from Enterobacter and Serratia (Blazevic, 1968). For convenience, these two important tests have been combined with sulphide-production in one tube.

The production of hydrogen sulphide is a useful diagnostic test in the identification of enteric bacteria and is helpful in the differentiation between Salmonella and Shigella. The sulphate reducing bacteria will produce hydrogen sulphide and further chemical substitution results in iron II sulphide being formed along the line of inoculation.

The presence of fermentable sugars may suppress the enzyme mechanism which forms hydrogen sulphide, as a result of the acid products formed (Bulmash & Fulton, 1964) and therefore sugars are not included in the medium. Oxoid SIM Medium can be used in conjunction with Triple Sugar Iron Agar (Oxoid CM277) to assess the ability of the culture to ferment lactose, sucrose and dextrose.

The production of indole from tryptophan is one of the diagnostic tests used in identifying enteric bacteria. For example, unless it is an unusual form, a Salmonella culture never produces indole from tryptophan in amounts detectable in usual tests.

Tryptone is incorporated into the medium since it is a tryptophan rich peptone, and after incubation, indole can be identified by a red dye complex reaction with one of several reagents, e.g. Kovac's reagent which consists of amyl alcohol, para-dimethylaminobenzaldehyde and concentrated hydrochloric acid (Harrigan & McCance, 1966).

The presence of glucose in the medium is avoided as recommended (Wilson & Miles, 1964). False positive indole reactions have been recorded when fermentation has occurred (Gillies, 1956).

The use of only 0.35% agar in the medium results in the production of a semi-solid medium, ideal for the examination of motility. Non-motile organisms will grow along the line of inoculation only, whereas motile species will grow away from it.

Oxoid SIM Medium (CM435) is therefore designed to determine three characteristics: hydrogen sulphide production, indole production and motility.

TECHNIQUE

The medium should be dispensed in tubes or bottles and when cool, inoculated once with a pure culture, by inserting a straight wire to about one third of the depth of the medium. If papers are used for the detection of indole, then these are wedged between the cotton wool plug or cap, and side of the container.

The inoculated media is incubated at 37° C for 18 hours or longer, if necessary, and examined for motility, hydrogen sulphide production and finally indole production from tryptophan.

To test for indole production:

1. Add 0.2 ml of Kovac's Reagent to the tube and allow to stand for 10 minutes. A dark red colour in the reagent constitutes a positive indole test: no change in the original colour of the reagent constitutes a negative test, or:
2. Suspend a strip of filter paper, soaked in a solution of saturated oxalic acid and dried over the medium (Wilson & Miles, 1964). Indole formed by positive organisms is volatile and causes the test paper to turn pink.

COLONIAL APPEARANCES

Non-motile organisms grow only along the line of inoculation, whereas motile species show either a diffuse even growth spreading from the inoculum, turbidity of the whole medium, or more rarely, localized outgrowths which are usually fan-shaped or occasionally nodular.

Hydrogen sulphide production is shown by blackening of the line of inoculation.

REFERENCES

Blazevic, D. J. (1968) 'Improved Motility Indole Medium' *Appl. Microbiol.* **16,** (4), 668.

Bulmash, J. M. and Fulton, M. (1964) 'Discrepant Tests for Hydrogen Sulphide' *J. Bact.* **88,** (6), 1813.

Gillies, R. R. (1965) 'An evaluation of two composite media for preliminary identification of Shigella and Salmonella' *J. clin. Path.* **9,** 368–371.

Harrigan, W. F. and McCance, M. E. (1966) 'Laboratory Methods in Microbiology' *Academic Press.* **53.**

Wilson, G. S. and Miles, A. A. (1964) Topley and Wilson's 'Principles of Bacteriology and Immunity' 5th ed. Arnold. **1,** 490.

Skim Milk Powder

Code L31

The use of ordinary skim milk powder is undesirable in bacteriological media because of the presence of heat-resistant organisms which give rise to erroneous cultural results. Oxoid Skim Milk Powder is a special bacteriological grade, consisting of thermophile-free spray-dried skim milk.

Average analysis:

Moisture	5.0%
Ash	8.0%
Total Nitrogen	5.3%
Reducing Sugars (as lactose monohydrate)	48.0%
Ether Soluble Extract	0.25%

Mix the powder to a smooth paste with a small quantity of distilled water, then gradually add more distilled water until a 10% w/v mixture is obtained—this is equivalent to fresh milk, and may be sterilized by autoclaving for 5 minutes at 121° C. Care should be taken not to overheat during sterilization, otherwise caramelization will occur. This product may be used alone or as a constituent of more complex bacteriological culture media. A 10% 'solution' of Skim Milk Powder, containing 0.004% of bromo-cresol purple forms a highly satisfactory purple milk which may be employed for the cultivation of dairy organisms or for the differentiation of *Clostridium* species, etc. Media containing skim milk powder are of particular value for diagnostic cultural tests involving the fermentation of lactose and digestion or coagulation of casein.

Slanetz and Bartley Medium

Code CM377

FORMULA

Tryptose (Oxoid L47)	*grams per litre*	**20.0**
Yeast Extract (Oxoid L21)		**5.0**
Dextrose		**2.0**
Disodium phosphate $2H_2O$		**4.0**
Sodium azide		**0.4**
Tetrazolium chloride		**0.1**
Agar No. 1 (Oxoid L11)		**10.0**

pH 7.2 (approx.)

DIRECTIONS

Suspend 42 grams in 1 litre of distilled water and bring to the boil to dissolve the agar completely. EXCESSIVE HEATING MUST BE AVOIDED. Dispense into petri dishes and allow to solidify. The medium should not be remelted, but poured plates enclosed in sealed containers, protected from light, can be kept at 4° C for up to 6 months.

DESCRIPTION

Slanetz & Bartley (1957) originally devised this medium to detect and enumerate faecal streptococci by the technique of membrane filtration, but it has also proved useful as a direct plating medium (Burkwall & Hartman, 1964, and the Nordic Committee on Food Analysis, 1968).

The medium is very selective for streptococci and, when it is incubated at elevated temperatures (44–45° C), all red or maroon colonies may be accepted as faecal streptococci (Taylor & Burman, 1964, and Mead, 1966).

Burkwall & Hartman showed that the addition of 0.5 ml of Tween 80 and 20 ml of a 10% solution of sodium carbonate or bicarbonate to each litre of medium was of value when examining frozen foods for streptococci; the original article should be consulted for procedural details.

TECHNIQUE

The Department of Health (1969) in their 'Report 71' recommend the use of Slanetz and Bartley medium for the enumeration of faecal streptococci in water supplies. The water is filtered through an Oxoid membrane filter which is then placed on the surface of a well dried plate of the medium. Plates are incubated at 37° C for 4 hours and then at 44–45° C for 44 hours. Membranes are examined, with a hand lens in a good light, and all red or maroon colonies counted as faecal streptococci.

Food samples can be examined for faecal streptococci by the method suggested by the Nordic Committee of Food Analysis (1968). Samples are homogenised and so diluted with physiological saline that only 15–150 colonies grow on each petri dish. Homogenates or dilutions are spread evenly over the agar surface with a glass rod and allowed to soak in. Dishes are inverted and incubated at 37° C for 48 hours, after which typical colonies (pink or dark red, with a narrow whitish border) are counted.

REFERENCES

Burkwall, M. K. and Hartman, P. A. (1964) Comparison of direct plating media for the isolation and enumeration of enterococci in certain frozen foods' *Appl. Microbiol.* 12, 18–23.

Department of Health and Social Security (1969) Report on public health and medical subjects No. 71. 'The bacteriological examination of water supplies' 4th edn. London. HMSO.

Mead, G. C. (1966) 'Faecal streptococci in water supplies and the problem of selective isolation' *Proc. Soc. Wat. Treat. Exam.* **15**, 207–221.

Nordic Committee on Food Analysis (1968) 'Tentative method for the determination of faecal steptococci in foods' *Leaflet* 68.

Slanetz, L. W. and Bartley, C. H. (1957) 'Numbers of enterococci in water, sewage and faeces determined by the membrane filter technique with an improved medium' *J. Bact.* **74**, 591–595.

Taylor, E. W. and Burman, N. P. (1964) The application of membrane filtration techniques to the bacteriological examination of water' *J. Appl. Bact.* **27**, 294–303.

Sodium Biselenite

Sodium hydrogen selenite

Code L121

For use in Oxoid Selenite Broth Base CM395/396 and Mannitol Selenite Broth Base CM399. CAP SECURELY AFTER USE.

Dissolve 4 grams in 1 litre of distilled water and use this solution to reconstitute the base medium or tablets.

Caution: Do not allow to remain in contact with the skin.

Sodium Chloride

Code L5

Also available as tablets—see page 238.

Sodium Thioglycollate

Code L120

For use in bacteriological culture media in order to lower their oxidation-reduction potential and to neutralize mercurial preservatives.

Spore Strips

Code BR23

DESCRIPTION

Biological tests for the adequacy of sterilization processes are not a new conception. Various methods have been described based on the cultivation of surviving organisms after subjecting them to the sterilizing process to be evaluated. Such tests, utilizing straw and other substances as a source of resistant organisms, may give dangerously misleading results due to variations in heat resistance. Even with spore preparations, certain organisms may be insufficiently heat-resistant to provide an adequate safety margin when used as controls, while others are too heat-resistant and lead to the needless rejection of some otherwise adequate sterilization procedures (Kelsey, 1958). In the same paper, Kelsey stressed the need for standard spore preparations of known heat resistance, ideally provided by a reference laboratory or some commercial source. The report of The Nuffield Provincial Hospitals Trust (1958) recommends the use of *Bacillus stearothermophilus* spores for the testing of autoclaves, but states that such controls must be of known thermal resistance, requiring careful and experienced preparation.

Oxoid Spore Strips fulfil the need for a dependable and positive index of sterilizing efficiency, offering many of the advantages of biological testing allied with extreme simplicity and convenience of operation. Each Oxoid Spore Strip is impregnated with viable spores of *B. stearothermophilus* and is contained inside a sealed glassine envelope. Heat resistance is carefully established in a high speed steam sterilizer (with minimal heating-up and cooling-down times) so that the spores survive 5 minutes at 121° C but are destroyed after 15 minutes at 121° C. Each batch of Spore Strips is also calibrated by plotting dose-response curves for exposure to steam (Kelsey, 1961).

TECHNIQUE

The use of *B. stearothermophilus* spores, in conjunction with Oxoid culture media for testing the sterilizing efficiency of autoclaves, is described in the report by the operational research team of the Nuffield Provincial Hospitals Trust (1958). A dressing drum approximately 12 inches diameter and 10 inches deep was employed, lined with two layers of butter muslin and packed with 18 dressing towels, each folded 2 × 2 × 3, so as to give 12 thicknesses of material. Spore preparations wrapped in one of the towels were inserted in the middle of the drum, and the drum was placed at the bottom of the autoclave, with the towel folds vertical, where sterilizing conditions were thought to be least favourable.

To test the efficiency of autoclaves and other sterilizing apparatus with the Oxoid Spore Strip, the Strip is subjected to the normal sterilization procedure and then cultivated. The precise technique of the test may be considered in two main stages as follows:

A. In the Sterilizing Room

(1) Remove a glassine envelope (containing 2 Spore Strips) from its paper envelope and place in a suitable position in the sterilizer, where sterilization conditions are likely to be least favourable. When dressings are being sterilized, a recommended position for the two strips is the centre of the largest bundle. If an autoclave is being tested, the bundle is then placed at the bottom and front of the chamber and processed with a full load of dressings.

(2) Record the relevant information on the front of the paper envelope.

(3) After 'sterilization' return the glassine wrapper containing the Spore Strips to its paper envelope, seal and forward to the laboratory for examination.

B. In the Laboratory

(1) Aseptically cut open the glassine wrappers and transfer each spore strip to 10 ml of sterile Oxoid Tryptone Soya Broth (CM129). Carry out a duplicate test on an unsterilized Spore Strip to act as a control.

(2) Incubate for 4 days at 50–60° C and observe for growth of the organism, shown by development of turbidity in the broth often observed after 48 hours. Positive cultures from the heated strips are evidence of ineffective sterilization. Negative cultures in the presence of a positive control are good evidence of effective sterilization.

QUALITY CONTROL

Oxoid Spore Strips are controlled for accurate performance according to the method of Kelsey (1961).

REFERENCES

Kelsey, J. C. (1958) 'The Testing of Sterilizers' *Lancet* **1**, 306–309.
Kelsey, J. C. (1961) 'The Testing of Sterilizers. 2. Thermophilic Spore Papers' *J. clin. Path.* **14**(3), 313–319.
Nuffield Provincial Hospitals Trust (1958) 'Present Sterilizing Practice in Six Hospitals' NPHT, London.

S.S. Agar

Salmonella Shigella Agar

Code—Powder CM99
Tablets CM100

FORMULA

'Lab-Lemco' Powder (Oxoid L29) *grams per litre*	**5.0**
Peptone (Oxoid L37)	**5.0**
Lactose	**10.0**
Bile Salts No. 3 (Oxoid L56)	**8.5**
Sodium citrate	**10.0**
Sodium thiosulphate	**8.5**
Ferric citrate	**1.0**
Brilliant green	**0.00033**
Neutral red	**0.025**
Agar No. 3 (Oxoid L13)	**15.0**

pH 7.0 (approx.)

DIRECTIONS

Powder: Suspend 63 g in 1 litre of distilled water. Bring to the boil with frequent agitation, and allow to simmer gently to dissolve the agar. DO NOT AUTOCLAVE. Cool to about 50° C, mix and pour into Petri dishes.
Tablets: Add 1 tablet to each 5 ml of distilled water and soak for 5 minutes. Bring to the boil in a water bath to dissolve the medium completely. Mix well before pouring. DO NOT AUTOCLAVE.

DESCRIPTION

SS Agar is a differential, selective medium for the isolation of *Shigella* and *Salmonella* species from pathological specimens, suspected foodstuffs, etc. Gram-positive and coliform organisms are inhibited by the action of a specially prepared Bile Salts mixture.

TECHNIQUE

Inoculate the medium heavily with the specimen, spreading a portion of the original inoculum in order to obtain well separated colonies on some part of the plate. Incubate for 18 to 24 hours at 37° C: non-lactose fermenters form colourless colonies, whilst occasional resistant coliforms or other lactose fermenters produce pink or red colonies.

In parallel with the SS Agar plate, inoculate a tube of Selenite Broth CM395 enrichment medium, incubate for 12 hours at 37° C, and subculture on to another SS Agar plate.

Standard Plate Count Agar

(A.P.H.A.)

Code—Powder CM463
Tablets CM464

FORMULA

Yeast Extract Powder (Oxoid L21) *grams per litre*	**2.5**
Pancreatic Digest of Casein (Oxoid L42)	**5.0**
Glucose	**1.0**
Agar No. 4 Powder (Oxoid L10)	**15.0**

pH 7.0 (approx)

DIRECTIONS

Powder: Suspend 23.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense into bottles and sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Standard Plate Count Agar (Oxoid CM463) conforms to the formulation for Standard Methods Agar, described in 'Standard Methods for the Examination of Dairy Products' 11th Edn. 1960.

It is the same formulation as that described for Plate Count Agar Dehydrated (Tryptone Glucose Yeast Agar) in the A.P.H.A. 'Standard Methods for the Examination of Water and Wastewater' 11th Edn. 1960. It is also the same formulation as the Tryptone Glucose Yeast Agar (U.S.P. XVI and A.O.A.C. 10th Edn. 1965).

The ingredients of Standard Plate Count Agar (Oxoid CM463) conform to the specifications quoted in the A.P.H.A. 'Standard Methods for the Examination of Dairy Products' 12th Edn. 1967, pp. 232–233.

The physical standards of the medium meet the specification described by the A.P.H.A. 12th Edn. 1967, p. 233.

The production of the medium is checked by the tests described in the above publication (pp. 234–242) where samples of raw and pasteurised milk are examined in parallel with the Standard Reference Medium.

Standard Plate Count Agar (Oxoid CM463) may also be used in the microbiological examination of foods as described by the Association of Official Agricultural Chemists 9th Edn. 1960 and 'Recommended Methods for Microbiological Examination of Foods' A.P.H.A. 1958.

Staphylococcus Medium No. 110

Code CM145

FORMULA

Yeast Extract (Oxoid L20)	*grams per litre*	2.5
Tryptone (Oxoid L42)		10.0
Lactose		2.0
Mannitol		10.0
Sodium chloride		75.0
Dipotassium hydrogen phosphate		5.0
Gelatin (Oxoid L8)		30.0
Agar No. 3 (Oxoid L13)		15.0

pH 7.1 (approx.)

DIRECTIONS

Suspend 150 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Disperse the precipitate by gentle agitation before pouring.

DESCRIPTION

Staphylococcus Medium No. 110 is a selective medium for isolation and differentiation of pathogenic staphylococci (Chapman, 1946) on a basis of salt tolerance, pigmentation, mannitol fermentation, and gelatin liquefaction. Pathogenic staphylococci (coagulase-positive) are able to grow on the high-salt mannitol medium to form orange colonies which give positive reactions for acid production, and gelatin liquefaction.

Stone (1935) suggested that gelatinase activity was indicative of food poisoning strains but Chapman *et al.* (1937) reported that typical food poisoning staphylococci should also produce an orange pigment, be haemolytic, be coagulase-positive, and ferment mannitol. Chapman (1947) showed that incubation at 30° C produced deeper pigmentation and no interference with the Stone reaction nor with acid production from mannitol —both of the latter being about as intense as at 37° C.

Smuckler & Appleman (1964) made Staphylococcus Medium No. 110 selective, for the determination of coagulase-positive staphylococci in meat pies containing large numbers of *Bacillus* organisms, by the addition of 0.75 mM of sodium azide.

TECHNIQUE

Streak or smear the Staphylococcus Medium No. 110 plate with the specimen and incubate for 43 hours at 37° C or for 48 hours at 30° C. Pigmented colonies are a deep orange colour, whilst non-pigmented colonies are white.

Acid production from mannitol is best demonstrated by adding a drop of 0.04% bromo thymol blue indicator to the sites of the individual colonies: yellow indicates acid production.

Gelatin hydrolysis may be demonstrated by adding a drop of a saturated aqueous solution of ammonium sulphate or, preferably, of a 20% aqueous solution of sulphosalicylic acid to an individual colony ('Stone reaction'). A positive 'Stone reaction' is denoted by the presence of a clear zone round gelatinase-producing colonies after 10 minutes' contact with the reagent.

The above reactions may be conveniently performed using short sleeves, 5 mm long and 10 mm diameter, cut from polythene tubing. The sleeves act as receptacles for the reagents when placed over discrete colonies, and may be stored in 70% alcohol prior to use.

Coagulase tests should not be carried out without first subculturing in Nutrient Broth No. 2 CM67 or on Blood Agar Base CM55.

REFERENCES

Chapman, G. H., Lieb, C. W. and Cumco, L. G. (1937) 'Isolation and Differentiation of Food-poisoning Staphylococci' *Food Research* **2,** 349–367.

Chapman G. H. (1946) 'A Single Culture Medium for Selective Isolation of Plasma-Coagulating Staphylococci and for Improved Testing of Chromogenesis, Plasma Coagulation, Mannitol Fermentation and the Stone Reaction' *J. Bact.* **51,** 409–410.

Chapman, G. H. (1947) 'Advantages of Incubation at 30° C for the Study of Staphylococci' *J. Bact.* **53,** 365–366.

Smuckler, S. A. and Appleman, M. D. (1964) 'Improved Staphylococcus medium No. 110' *Appl. Microbiol.* **12,** 355–359.

Stone, R. V. (1935) 'A Cultural Method for Classifying Staphylococcus as of the "Food-poisoning" type' *Proc. Soc. Exper. Biol. & Med.* **33,** 185–187.

Stuart Transport Medium (MODIFIED)

Code—Powder CM111
Tablets CM112

FORMULA

	grams per litre
Sodium glycerophosphate	**10.0**
Sodium thioglycollate	**0.5**
Cysteine hydrochloride	**0.5**
Calcium chloride	**0.1**
Methylene blue	**0.001**
Agar No. 1 (Oxoid L11)	**10.0**

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 21 g in 1 litre of distilled water. Bring to the boil to dissolve completely and dispense into screw-capped ¼ oz. 'Bijou' bottles. Fill each bottle to the brim, tighten the cap and sterilize by autoclaving at 121° C for 15 minutes. When sufficiently cool to handle, mix by inversion.
Tablets: Place 1 tablet in a screw-capped ¼ oz. 'Bijou' bottle, fill to the brim with distilled water and apply the cap tightly. Allow to soak for 5 minutes and sterilize by autoclaving at 121° C for 15 minutes. Mix by inversion when cool.

A blue coloration of the sterilized medium indicates that it is aerated and consequently, unfit for use. The air present may be easily removed by re-steaming, with the screw cap loosened.

DESCRIPTION

This improved medium originally described by Moffett *et al.* (1948) and Stuart *et al.* (1954), is a non-nutritional semi-solid substrate for the preservation of *Neisseria* species and other fastidious organisms during their transport from clinic to laboratory. Originally formulated for the conservation of *Neisseria gonorrhoeae* and *Trichomonas vaginalis*, it may also be used for the transport of other bacteriological specimens. Stuart *et al.* (1954) noted that the transport medium may also be used for *Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes*, and *Corynebacterium diphtheriae*. Cooper (1957) investigated the extension of Stuart's method to the transport of swabs of clinical material containing upper respiratory tract and enteric pathogens. Stuart (1959) published an account of his experiences of the medium in public health bacteriology, whilst Crookes & Stuart (1959) used the transport method in combination with cultivation for *N. gonorrhoeae* on an Aerosporin-Peizer medium.

Preparation of Charcoal Swabs for use with Transport Medium.

(1) Prepare swabs by rolling absorbent cotton-wool on wooden* sticks.

(2) Boil the swabs in a phosphate buffer solution of the following composition:

Disodium hydrogen phosphate	0.81 grams
Potassium dihydrogen phosphate	0.18 grams
Distilled water	100 ml

pH 7.4

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* Beakley (1957) recommended aluminium due to the toxicity of wood towards *Neisseria gonorrhoeae.*

(3) Immediately dip the swabs into a 1% suspension of Charcoal Bacteriological (Oxoid L9).

(4) Place in cotton-wool plugged test tubes, dry at 100° C and sterilize in the hot air oven for 1 hour at 160° C.

Transport of Swabs:

(5) After collection of the specimen, place the swab in the middle of a 'bijou' bottle of Stuart Transport Medium. Break off the stick, replace the screw cap tightly and store the bottle in a cool place.

The transport method will allow the isolation of gonococci from approximately 90% of cases of female gonorrhoea, provided the transport period is under 24 hours; for longer periods the method is still useful up to 3 days (Stuart *et al.*, 1954). In all cases, specimens should be cultivated as soon as possible or stored in the refrigerator if delay is unavoidable. Wilkinson (1958) reported successful isolation after as long as six days' storage in a refrigerator.

Trichomonas vaginalis remains viable, in the medium, up to 24 hours whilst Cooper (1957) has reported the recovery of upper respiratory tract and enteric pathogens after 8 to 12 weeks' storage. Stuart *et al.* (1954) successfully used the transport method for the recovery of *H. influenzae, Str. pneumoniae, Str. pyogenes,* and *C. diphtheriae* from specimens which had been in transit for 3 to 5 days.

REFERENCES

Beakley, J. W. (1957) 'The Toxicity of Wooden Applicator Sticks for *Neisseria gonorrhoea. Pub. Hlth Lab.* **15**(1), 11–16.

Cooper, G. N. (1957) 'The Prolonged Survival of Upper Respiratory Tract and Intestinal Pathogens on Swabs' *J. clin. Path.* **10**(3), 226–230.

Crookes, Eileen M. L. and Stuart, R. D. (1959) 'The Value of Aerosporin in the isolation of *Neisseria* from Swabs forwarded to the Laboratory in Transport Medium' *J. Path. Bact.* **78**(1), 283–288.

Moffett, Mary, Young, Jean L. and Stuart, R. D. (1948) Centralized Gonococcus Culture for Dispersed Clinics' *Brit. Med. J.* **2**, 421–424.

Stuart, R. D., Toshach, Sheila R. and Patsula, Teresa M. (1954) 'The Problem of Transport of Specimens for Culture of Gonococci' *Canad. J. Publ. Hlth* **45**(2), 73–83.

Stuart, R. D. (1959) 'Transport Medium for Specimens in Public Health Bacteriology' *Pub. Hlth Rep. Wash.* **74**(5), 431–438.

Wilkinson, A. E. (1958) 'Some Recent Advances in the Laboratory Diagnosis of Venereal Disease' *J. Med. Lab. Technol.* **15**(3), 184–195.

Sucrose 10%

SR13

Filter sterilized solution, available in 5 ml ampoules, in boxes of ten.

Sugar Free Agar

Code CM417

FORMULA

	grams per litre
Gelatin Peptone	**7.5**
Tryptone (Oxoid L42)	**7.5**
Sodium chloride	**5.0**
Agar No. 3 (Oxoid L13)	**14.0**

pH 7.6 (approx)

DIRECTIONS

Suspend 34 g of medium in 1 litre of distilled water. Boil to dissolve and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Oxoid Sugar Free Agar is made according to a standard formula (International Dairy Federation, 1964) for the detection and enumeration of contaminating organisms in butter and other processed dairy products.

This agar contains two peptones free of carbohydrates, as devised by Ritter and Eschmann (1966). Psychrotrophic and mesophilic gram-negative, rod-shaped bacteria develop well on Sugar Free Agar as they are able to deaminate protein as a carbon source.

The formulation of Sugar Free Agar prevents the interference of some Lancefield group D streptococci in a total count of gram-negative, rod-shaped bacteria (Thomas, 1969; Mossel, Krol and Moerman, 1972). It is thus useful for the differentiation of non-lactic acid contaminating micro-organisms from lactic acid bacteria.

TECHNIQUE

1. Dilute a 2.5 g sample with 5 ml ¼-strength Ringers solution (Oxoid BR52).
2. Pour plates with 0.2 ml of the sample or aliquots of further dilutions if necessary.
3. Incubate for two days at 30° C and a further two days at 20° C. See FIL-IDF 30 for further details.

REFERENCES

International Dairy Federation (1964) International Standard FIL-IDF 30.
Mossel, D. A. A., Krol, B. and Moerman, P. C. (1972) 'Bacteriological and Quality Perspectives of Salmonella Radicidation of Frozen, Boneless Meats.' *Alimenta* **11**, (2), 51–60.
Ritter, P. and Eschmann, K. H. (1966) 'Bakteriologische Prufung und Beurteilung von Tafelbutter.' *Alimenta* **5**, (2), 43–45.
Thomas, S. B. (1969) 'Methods of assessing the psychrotrophic bacterial content of milk.' *J appl. Bact.*, **32**, 269–296.

T.C.B.S. Cholera Medium

Code CM333

FORMULA

	grams per litre
Yeast Extract Powder (Oxoid L21)	**5**
Bacteriological Peptone (Oxoid L34)	**10**
Sodium thiosulphate	**10**
Sodium citrate	**10**
Ox Bile	**8**
Sucrose	**20**
Sodium chloride	**10**
Ferric citrate	**1**
Brom-thymol blue	**0.04**
Thymol-blue	**0.04**
Agar No. 1 (Oxoid L11)	**14**

pH 8.6 (approx.)

DIRECTIONS

Suspend 88 grams in 1 litre of distilled water. Boil to dissolve the medium completely. DO NOT AUTOCLAVE.

Pour plates without further heating and dry before use.

DESCRIPTION

Kobayashi, Enomoto, Sakazaki and Kuwahara (1963) developed T.C.B.S. media from the selective isolation agar of Nakanishi (1963).

The Oxoid T.C.B.S. medium conforms to the formulation of Kobayashi *et al.*, except that it contains specially processed ox bile, free from the defects noted by Nakanishi and Kobayashi.

The Oxoid medium is particularly suitable for the growth of Vibrio cholerae biotype el tor and V. parahaemolyticus, in addition to the classical V. cholerae.

Sakazaki (1963) described the isolation of V. parahaemolyticus and V. alginolyticus on T.C.B.S. medium.

Most of the Enterobacteriaceae encountered in faeces are totally suppressed for at least 24 hours. Slight growth of *Proteus spp.* and *Strept. faecalis* may occur but the colonies are easily distinguished from vibrio colonies.

Oxoid T.C.B.S. medium is complete and requires no additives or aseptic additions of blood. It therefore shows a considerable advantage over Lauryl Sulphate Tellurite Agar which requires further additions after sterilization. Apart from this convenience factor, it also possesses superior growth characteristics for *Vibrio spp.*, compared with tellurite media. Whilst inhibiting non-vibrios, it promotes the rapid growth of *Vibrio spp.*, after overnight incubation at 37° C.

COLONIAL APPEARANCE ON T.C.B.S. AGAR

After 24 hours incubation at 37° C.

V. cholerae—yellow-brown colonies, 2–3 mm diameter, yellow colour in medium.

V. parahaemolyticus (Group 1)—colourless colonies with green centre, 3–4 mm in diameter.

V. parahaemolyticus (Group 2)—yellow-brown colonies, 3–4 mm diameter, yellow colour in medium.

Escherichia coli *Salmonella typhi* *Klebsiella spp.* *Shigella spp.* *Pseudomonas aeruginosa* *Proteus spp.*	Slight growth with minute colonies. No yellow colour in medium.

Streptococcus faecalis—slight growth with minute colonies. Yellow colour in medium.

TECHNIQUE

Streak the faeces or other material under investigation across the surface of Oxoid T.C.B.S. Cholera Medium and incubate for 18–24 hours at 37° C.

All *V. cholerae* strains produce large smooth, raised yellow coloured colonies with yellow colour in the medium.

V. parahaemolyticus strains produce similar colonies, with the exception of Group 1 organisms which produce green colonies without altering the colour of the medium.

REFERENCES

Sakazaki, R. (1963) Vibrio parahaemolyticus Tokyo, Osseido.
Nakanishi, Y. (1963) 'An Isolation Agar Medium for Cholera and Enteropathogenic Halophilic Vibrios' *Modern Media* **9**, 246.
Kobayashi, T., Enomoto, S., Sakazaki, R. and Kuwahara, S. (1963) 'A New Selective Medium for Pathogenic Vibrios: T.C.B.S. Agar (Modified Nakanishi's Agar)' *Jap. J. Bacteriol.* **18**, 10–11, 387–391.

Tellurite Medium Hoyle

See page 139.

Test Tube Caps (ALUMINIUM)

Cylindrical aluminium caps which are used to provide a bacteriological closure for test tubes, bottles and flasks. For this purpose, Oxoid Test Tube Caps have many advantages over the conventional cotton-wool plugs:

(i) the caps can be used repeatedly;

(ii) they will not stick to the neck of the container, and are easily removed and replaced;

(iii) there are no fibres to contaminate media—a factor of considerable importance in studies of bacterial nutrition and in microbiological assay work;

(iv) the caps may be easily marked with a pencil, or with paint which will withstand autoclaving;

(v) sterility is ensured for a long period, although caps may be fixed to the container with 'Sellotape' if necessary.

Oxoid Test Tube-Caps are supplied to fit 12 mm (½-in.), 16 mm (⅝-in.), 19 mm (¾-in.), and 25 mm (1-in.) British Standard rimless test tubes, and the 30 mm (1¼-in.) size will fit a 3-oz. milk sampling bottle. In addition John Moncrieff Ltd. of Perth make a range of 'Monax' conical flasks which may be closed with Oxoid Caps.

To assist identification, Oxoid Test Tube Caps are supplied in the following anodized colours:

Green, Blue, Black, Silver, Mauve, Gold, Copper, Red.

It is particularly important to avoid strong alkalis and abrasives when cleaning the coloured or plain caps. Preferably, wash in warm water containing a small quantity of non-alkaline detergent (e.g. 'Teepol', 'Tide', 'Quix', etc.), rinse thoroughly in warm water, and dry in an oven at 100–120° C.

Test Tube Caps have long been an accepted feature of many bacteriological laboratories. The following is an extract from Ministry of Health Report No. 71 (1939—Reprinted 1954) 'The Bacteriological Examination of Water Supplies':

> 'All tubes should be plugged with cotton-wool or covered with closely fitting aluminium caps . . . These caps have the great advantage that they can be removed and replaced by the fingers of one hand, leaving the other hand free to manipulate a pipette or wire loop. They can be used over and over again, and therefore effect a great economy in cotton-wool. Test tubes of media covered with these caps remain sterile indefinitely.'

Oxoid Test Tube Caps have been used for a number of purposes (see the references), especially for the closure of tubes of media used in growth, nutritional and physiological studies (Rogosa *et al.*, 1961; Bachrach, 1957; Painter, 1954; Hagen & Rose, 1961; Hugo & Rogers, 1958). Hart *et al.* (1962) used Oxoid Test Tube Caps in order to avoid the toxic effect of cotton-wool on tubercle bacilli.

Due to their attractive appearance and the range of colours in which they are available, Oxoid Test Tube Caps are also especially suitable for exhibition and demonstration purposes (McCormick, 1955–56).

REFERENCES

Bachrach, U. (1957) 'The Aerobic Breakdown of Uric Acid by Certain Pseudomonads' *J. gen. Microbiol.* **17**(1), 1–11.
Beech, F. W., Carr, J. G. and Codner, R. C. (1955) 'A Multipoint Inoculator for Plating Bacteria or Yeasts' *J. gen. Microbiol.* **13**(3), 408–410.
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Hagen, P.-O. and Rose, A. H. (1961) 'A Psychrophilic Cryptococcus' *Can J. Microbiol.* **7**(3), 287–294.
Hart, P. D'Arcy, Lovelock, J. E. and Nash, T. (1962) 'The Lethal Effect of Cotton-Wool Lipid on Tubercle Bacilli in Acid Conditions and its Prevention by Surface-Active Agents' *J. Hyg.* Camb. **60**(4), 509–525.
Hugo, W. B. and Rogers, Margaret K. (1958) 'The Nutrition and some Biochemical Studies of *Vibrio cyclosites* and *Vibrio neocistes*' *J. appl. Bact.* **21**(1), 20–25.
McCormick, W. (1955–56) 'Preservation of Some Bacteriological Tests and Specimens' *J. Med. Lab. Technol.* **13**, 487–488.
Newton, B. A. (1955) 'A Fluorescent Derivative of Polymyxin: its Preparation and Use in Studying the Site of Action of the Antibiotic' *J. gen. Microbiol.* **12**(2), 226–236.
Painter, H. A. (1954) 'Factors affecting the Growth of some Fungi associated with Sewage Purification' *J. gen. Microbiol.* **10**(1), 177–190.
Rogosa, M., Franklin, J. G. and Perry, K. D. (1961) 'Correlation of the Vitamin Requirements with Cultural and Biochemical Characters of *Lactobacillus* spp.' *J. gen. Microbiol.* **25**(3), 473–482.

Test Tubes Caps (POLYPROPYLENE)

These new caps, made of tough flexible polypropylene will withstand boiling and autoclaving and are resistant to most laboratory agents.

They are available in plain white and in 9 colours: Black, Blue, Brown, Green, Grey, Pink, Purple, Red and Yellow.

Each cap has two small internal projections, to ensure a firm fit on the test tube, and the recessed top may be labelled with pencil or grease pencil.

There are two sizes of cap:

Code T.T.P.2.—to fit the 16 mm (⅝-in.) British Standard Test Tube (B.S.S. 625: 1960).

Code T.T.P.6.—to fit the 17–18 mm standard test tube commonly used in Europe.

Tetrathionate Broth Base

Code—Powder CM29
Tablets CM30

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**0.9**
Peptone (Oxoid L37)		**4.5**
Yeast Extract (Oxoid L20)		**1.8**
Sodium chloride		**4.5**
Calcium carbonate		**25.0**
Sodium thiosulphate		**40.7**

DIRECTIONS

Powder. Add 77 g to 1 litre of distilled water and bring to the boil. Cool to below 45° C and add 20 ml of Iodine solution. Mix well and tube in 10 ml quantities. The sterilized base will keep for many weeks but should be used within a few hours of the addition of the Iodine solution.

Tablets: Add 2 tablets to 10 ml of distilled water. Bring to the boil, cool to below 45° C and add 0.2 ml of Iodine Solution.

Iodine Solution

Iodine	6 grams
Potassium Iodide	5 grams
Distilled Water	20 ml

DESCRIPTION

Tetrathionate Broth is recommended for the selective enrichment method of isolating *Salmonella typhosa* and other salmonellae from faeces, sewage, etc.

Organisms which reduce tetrathionate, such as salmonellae, flourish in the medium whilst many faecal organisms are inhibited (Knox *et al.*, 1942). Members of the *Proteus* group reduce tetrathionate and may consequently impair the value of this medium for the isolation of salmonellae; this disadvantage of the medium is largely overcome by the addition of 40 µg of Novobiocin to each millilitre of the incomplete medium before the addition of iodine (Jeffries, 1958; Buttiaux *et al.*, 1961).

TECHNIQUE

Inoculate the broth with about 2 grams of the specimen and mix thoroughly to disperse particulate matter. A loose cotton-wool plug may be passed down through the inoculated medium in order to carry gross particles to the bottom of the tube. Incubate for 12 to 24 hours at 37° C and then subculture on Bismuth Sulphite Agar CM201, SS Agar CM99 or Desoxycholate Citrate Agar (Hynes) CM227, etc.

The complete medium (with added iodine) should be used the same day as it is prepared, but the sterilized basal medium will keep for many weeks. Jeffries (1959) showed that Novobiocin, at a concentration of 40 µg/ml in the medium, remained stable for at least 48 hours at 37° C, and for one month at room temperature.

This medium is frequently used in parallel with Selenite Broth Base CM395.

REFERENCES

Buttiaux, R., Catsaras, M. and Verdant, M. (1961) 'Tetrathionate—Novobiocin Medium for Enrichment of *Salmonella* Isolated from Faeces' *Ann. Inst. Pasteur de Lille* **12,** 13–18.

Jeffries, L. (1959) 'Novobiocin—Tetrathionate Broth: A Medium of Improved Selectivity for the Isolation of Salmonellae from Faeces' *J. clin. Path* **12**(6), 568–571.

Knox, R., Gell, P. G. H. and Pollock, M. R. (1942) 'Selective Media for Organisms of the *Salmonella* Group' *J. Path. Bact.* **54,** 469–483.

Thioglycollate Broth U.S.P. — Alternative

Code CM391

FORMULA

L-cystine	*grams per litre*	**0.5**
Sodium chloride		**2.5**
Dextrose		**5.5**
Yeast Extract (Oxoid L21)		**5.0**
Pancreatic Digest of Casein (Oxoid L42)		**15.0**
Sodium thioglycollate		**0.5**

pH 7.1 (approx.)

DIRECTIONS

Suspend 29 grams in 1 litre of distilled water. Bring to the boil and dissolve the medium completely. Distribute into tubes or bottles and sterilize by autoclaving at 121° C for 15 minutes.

PREPARE FRESHLY OR BOIL AND COOL THE MEDIUM JUST BEFORE USE.

DESCRIPTION

Thioglycollate Medium U.S.P.—Alternative, is intended for sterility testing with certain biological products that are turbid or otherwise do not lend themselves readily to culturing in Fluid Thioglycollate Medium U.S.P. (CM173) because of its viscosity.

The formulation, which omits the agar and resazurin present in Fluid Thioglycollate Medium U.S.P., is described in the N.I.H. Memorandum (1955) and the U.S. Pharmacopeia 18th Edn. (1970). These omissions make it essential that the medium should be freshly prepared or boiled and cooled within four hours of use.

The containers of choice for the medium are tubes of 20 × 150 mm size. The use of 15 ml of medium in this tube provides adequate medium for inocula up to 3 ml and sufficient thioglycollate to inactivate a mercurial preservative, when present in the inoculum, in a concentration not greater than 0.03% w/v. Other preservatives will need to be inactivated by adequate dilution unless an effective inactivator is used. (See Oxoid Clausen Medium CM353).

If larger volumes of medium are required, cylindrical or square bottles or containers which provide approximately the same ratio of surface exposed to depth of medium as in the test-tube above, should be used.

Whilst storage of the medium is not recommended, autoclaved volumes of Thioglycollate Medium U.S.P.—Alternative, should be held at 20°–30° C. Lower temperatures than these, increase the absorption of oxygen from the atmosphere.

REFERENCES

N.I.H. (1955) Memorandum: 'Culture media for sterility tests' 4th Revision.
U.S. Pharmacopeia (1970) 'Sterility tests', 18th Edn., pp. 851–852.

Thioglycollate Medium (BREWER)

Code—Powder CM23
Tablets CM24

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**1.0**
Yeast Extract (Oxoid L20)		**2.0**
Peptone (Oxoid L37)		**5.0**
Dextrose		**5.0**
Sodium chloride		**5.0**
Sodium thioglycollate		**1.1**
Methylene blue		**0.002**
Agar No. 1 (Oxoid L11)		**1.0**

pH 7.2 (approx.)

DIRECTIONS

Powder: Suspend 20 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 10 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes. Media containing small quantities of agar are liable to separate if cooled rapidly. Tubes of reconstituted and autoclaved medium should be allowed to cool slowly on a wooden surface in a draught-free atmosphere.

DESCRIPTION

This anaerobic medium is used principally for testing the sterility of biological products.

The medium contains a small concentration of methylene blue as an oxidation-reduction indicator. If more than 20% of the uppermost portion of the stored medium has changed to a green colour, anaerobic conditions may be restored by heating in a boiling water bath or steamer for 5 to 10 minutes. This treatment must not be repeated. The prepared medium should be stored in the dark at room temperature.

Thioglycollate Medium (Brewer) is especially useful for the control of biological solutions containing mercurial preservatives, the toxicity of the latter being neutralized by the thioglycollate.

Thioglycollate Medium USP

Code—Powder CM173
Tablets CM174

FORMULA

Yeast Extract Powder (Oxoid L21)	*grams per litre*	**5.0**
Tryptone (Oxoid L42)		**15.0**
Dextrose		**5.5**
Sodium thioglycollate		**0.5**
Sodium chloride		**2.5**
L-cystine		**0.5**
Resazurin		**0.001**
Agar No. 1 (Oxoid L11)		**0.5**

pH 7.1 (approx.)

DIRECTIONS

Powder: Suspend 29.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 to 18 minutes. Mix well and cool to approximately 25° C. If more than 20% of the medium is pink (due to oxidation) restore anaerobic conditions by reheating for 10 minutes in boiling water or steam. Do not reheat more than once.

Tablets: Add 1 tablet to 10 ml of distilled water and soak for 5 minutes. Autoclave at 121° C for 15 to 18 minutes. Mix well and cool to approximately 25° C.

DESCRIPTION

This medium is prepared according to the formula specified in the U.S. Pharmacopeia 18 (1970), for the performance of sterility tests. It is suitable for the cultivation of both aerobic and anaerobic organisms. No paraffin or special seal is necessary, nor is an anaerobic jar required for the cultivation of anaerobes. It is well buffered so that acid or alkaline inocula produce negligible alteration in the reaction of the medium. The sodium thioglycollate content of the medium will neutralize the bacteriostatic effect of mercurial compounds used as preservatives in solutions for injection, etc. If the solution undergoing test contains a bacteriostatic substance it is necessary, in order to avoid a false negative result, to establish the bacteriostatic activity of the product by the method described in the U.S. Pharmacopeia (1970). Thioglycollate Medium U.S.P. is also recommended for the cultivation of *Clostridium* species. Sealey (1951) found that, of the media tested, U.S.P. Fluid Thioglycollate gave the best results for the cultivation and maintenance of *Clostridium nigrificans*.

REFERENCES

Sealey, J. Q. (1951) *Thesis of the University of Texas.*
'The United States Pharmacopeia: Eighteenth Revision (1970), p. 852.

Todd-Hewitt Broth

Code CM189

FORMULA

Infusion from 450 g fat-free minced beef	*grams per litre*	**10.0**
Tryptone (Oxoid L42)		**20.0**
Dextrose		**2.0**
Sodium bicarbonate		**2.0**
Sodium chloride		**2.0**
Disodium phosphate amhyd.		**0.4**

pH 7.8 (approx.)

DIRECTIONS

Dissolve 36.4 g in 1 litre of distilled water. Mix well, distribute into containers and sterilize by autoclaving at 115° C for 10 minutes.

DESCRIPTION

An easily reconstituted, dehydrated modification of the medium originally described by Todd and Hewitt (1932) for the production of antigenic streptococcal haemolysin. Fermentation of dextrose, which is included as a growth stimulant, would lead to the destruction of haemolysin by the acid produced; consequently, the medium is buffered with sodium bicarbonate and sodium phosphate.

It has been found that inorganic phosphates have a stimulating effect on the growth of pneumococci quite apart from their buffering power.

Todd-Hewitt Broth may be employed as an alternative to serum broth or horse-flesh digest broth, for the cultivation of streptococci prior to serological grouping (Mackie and MacCartney, 1956).

REFERENCES

Mackie, T. J. and MacCartney, J. E. (1956) 'Handbook of Practical Bacteriology', 9th ed. (revised), Livingstone, London, p. 344.
Todd, E. W. and Hewitt, L. F. (1932) 'A New Culture Medium for the Production of Antigenic Steptococcal Haemolysin' *J. Path. Bact.* **35**(1), 973–974.

Tomato Juice Agar

Code CM113

FORMULA

Tomato juice (solids from 400 ml)	*grams per litre*	**20**
Peptone (Oxoid L37)		**10**
Peptonized Milk (Oxoid L32)		**10**
Agar No. 3 (Oxoid L13)		**12**

pH 6.1 (approx.)

DIRECTIONS

Suspend 52 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This is a modification of Kulp's medium for the culture of lactobacilli, and its preparation only differs slightly from the method described by Kulp & White (1932). Tomato Juice Agar is recommended for the cultivation and enumeration of *Lactobacillus* species.

When a more acid medium is required, for the direct plate count of lactobacilli and other organisms from saliva, the reaction of the medium may be adjusted to approximately pH 5.1 by the addition of 1 ml of 10% Lactic Acid SR21 to each 100 ml of sterilized medium.

Davis (1959) compared the morphology of *Lactobacillus* colonies on Oxoid Tomato Juice Agar and other media.

REFERENCES

Davis, G. H. G. (1959) 'Morphology and Bacterial Taxonomy' *Lab. Prac.* **8**(5), 161–167.
Kulp, J. W. L. and White, V. (1932) 'A Modified Medium for Plating *L. acidophilus*' *Science* **76**, 17–18.

Tributyrin Agar

Code PM4

FORMULA

Peptone (Oxoid L37)	*grams*	**5**
Yeast Extract Powder (Oxoid L21)		**3**
Tributyrin (glyceryl tributyrate)		**10**
Agar No. 3 (Oxoid L13)		**15**

and distilled water to 1 litre
pH 7.5 (approx.)

DESCRIPTION

Tributyrin Agar is used for the detection and enumeration of lipolytic (fat-splitting) bacteria and moulds. This medium, which consists of a stable homogenate of nutrient agar and tributyrin (glyceryl tributyrate), is recommended in 'British Standard No. 895—1940' for the differential enumeration of lipolytic organisms in butter. B.S. 895—1940 is reproduced in Tanner's 'The Microbiology of Foods'.

The Oxoid medium is also frequently used for the detection of the lipolytic activity of specific organisms such as staphylococci, clostridia, pseudomonads and flavobacteria—see the references.

TECHNIQUE

For use, it is normally sufficient to prepare 1/10, 1/100, 1/1,000 and 1/10,000 homogenates of the melted butter in quarter-strength Ringer

Solution and, within 10 minutes, to transfer 1 ml quantities of each dilution to separate sterile Petri dishes. Add 10 ml of the medium, melted and cooled to 45–48° C, mix, and incubate at 30° C for three days. After incubation, the medium appears opaque but lipolytic colonies are surrounded by a zone of clear medium.

B.S. No. 895—1940 states: 'There is evidence that a high proportion of organisms splitting tributyrin also split butter fat. As the rate of zone production in tributyrin is much faster than in butter fat the former has been adopted, as it permits the results to be obtained more quickly.'

REFERENCES

British Standards Institution (1940) 'British Standard Methods for the Microbiological Examination of Butter' B.S. No. 895–1940. HMSO, London.

Davey, B. B. and Turner, Myfanwy (1961) 'Some Phenol Decomposing Strains of *Pseudomonas*' *J. appl. Bact.* **24**(1), 78–82.

Hayes, P. R. (1963) 'Studies on Marine Flavobacteria' *J. gen. Microbiol.* **30**(1), 1–19.

Innes, A. G. (1956) 'Coagulase Positive Staphylococci from Bulk Milk Supplies Low in Solids-not-fat' *J. appl. Bact.* **19**(1), 39–45.

Tanner, F. W. (1944) 'The Microbiology of Foods', 2nd ed., Garrard Press, Illinois, pp. 474–479.

Willis, A. T. (1960) 'The Lipolytic Activity of some Clostridia' *J. Path. Bact.* **80**(2), 379–390.

Willis, A. T. and Gowland, G. (1960) 'Egg-Yolk Reactions of *Pseudomonas* species' *Nature* **187**(4735), 432–433.

Trichomonas Medium

Code CM161

FORMULA

Liver Digest (Oxoid L27)	*grams per litre*	**25.0**
Sodium chloride		**6.5**
Dextrose		**5.0**
Agar No. 1 (Oxoid L11)		**1.0**

pH 6.4 (approx.)

DIRECTIONS

Suspend 37.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Cool to 50° C. Inactivate 80 ml of horse serum*, adjust to pH 6.0 and add it to the medium. For diagnostic work, bacterial growth may be suppressed by the addition of 1,000 units of penicillin and 500 mcg of streptomycin per ml of medium.

DESCRIPTION

A medium based on that of Feinberg & Whittington (1957) for the detection of *Trichomonas vaginalis* and *Candida* species. The authors, after a series of investigations on 1,704 genito-urinary specimens, found that *T. vaginalis* would not have been detected in 23.5% of the samples had they not been cultured. Furthermore, out of 747 vaginal specimens, 63% which were positive would have been dismissed as negative without use of the medium.

Stenton (1957) noted that the success of an earlier version of the Trichomonas medium was largely dependant on the choice and percentage of liver incorporated; after the most exhaustive experiments he selected Oxoid Liver Infusion. Trichomonas Medium has been slightly modified by the incorporation of 0.1% w/v of 'Ionagar' No. 2, this leads to reduced oxygen tension and consequently much more prolific growth of trichomonads.

This medium, with added antibiotics, keeps well at + 4° C to + 5° C and there is no deterioration in antibacterial power after 3 months' storage. A good growth of both *Trichomonas* and *Candida* may be obtained in mixed culture—the growth of *Candida* species does not interfere with that of *Trichomonas.*

TECHNIQUE

Inoculate Trichomonas Medium and incubate at 36° C for three to five days. At intervals, microscopically examine a loopful of the culture. In addition, microscopically examine fresh wet smears of the specimen at the time of initial culture.

The medium is equally suitable for the examination of urethral and vaginal swabs, and urine specimens.

REFERENCES

Feinberg, J. G. and Whittington, Joan M. (1957) 'A Culture Medium for *Trichomonas vaginalis Donné* and Species of *Candida*' *J. clin. Path.* **10**(4), 327–329.

Stenton, P. (1957) 'The Isolation of *Trichomonas vaginalis*' *J. Med. Lab. Technol.* **14**(4) 228–230.

Trichomonas Medium No. 2

Code R27

FORMULA

Liver Digest (Oxoid L27)	*grams per litre*	**18.0**
Dextrose		**20.0**
Chloramphenicol		**0.125**
Tryptone Soya Broth (Oxoid CM129)		**30.0**
Horse Serum (Oxoid SR35)		**250 ml**
Calcium pantothenate 0.5% w/v		**1 ml**
Distilled water		**1,000 ml**

pH 6.2 (approx.)

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* Prior to incorporation in the medium, Horse Serum (Oxoid SR35) is inactivated by maintaining at a temperature of 56° C for 30 minutes, and then acidified with N/1 hydrochloric acid to pH 6.0.

DESCRIPTION

A modification of the media described by Bushby and Copp (1955) and Squires and McFadzean (1962) for the isolation of *Trichomonas vaginalis*. Trichomonas Medium No. 2 is one of the special new range of Lab-Ready Media which are supplied in glass ¼-oz. bottles, ready for immediate use.

Clinical trials have established that Trichomonas Medium No. 2 is very successful for the culture of *Trichomonas vaginalis* from primary isolates. A combination of laboratory culture and slide examination provides the best chance of establishing the presence of this pathogen.

Trichomonas Medium No. 2 contains both serum and antibiotic and is therefore ready for immediate inoculation. It will store at 4° C for several months without deterioration.

TECHNIQUE

1. Inoculate the medium directly with vaginal swab, sub-preputial scrape, urethral scrape, prostatic fluid, or the deposit from lightly centrifuged urine (Stenton, 1957).

2. Incubate at 34° C (Thomas, 1964).

3. Examine microscopically every 24 hours.

4. Incubate microscopically negative cultures up to 5 days.

REFERENCES

Bushby, S. R. M. and Copp, F. C. (1955) 'The Antitrichomonal Activity of Amidonitrothiazoles' *J. Pharm. Pharmacol.* **7**, 112–117.

Squires, S. and McFadzean, J. A. (1962) 'Strain Sensitivity of *Trichomonas vaginalis* to Metronidazole' *Brit. J. vener. Dis.* **38**, 218–219.

Stenton, P. (1957) 'The Isolation of *Trichomonas vaginalis*' *J. Med. Lab. Technol.* **14(4)** 228–230.

Thomas, Patricia M. (1964) 'Some Laboratory Aspects of *Trichomonas vaginalis*' *J. Med. Lab. Technol.* **21**(1), 46–50.

Triple Sugar Iron Agar

Code—Powder CM277
Tablets CM278

FORMULA

	grams per litre
'Lab-Lemco' Powder (Oxoid L29)	**3.0**
Yeast Extract (Oxoid L20)	**3.0**
Peptone (Oxoid L37)	**20.0**
Sodium chloride	**5.0**
Lactose	**10.0**
Sucrose	**10.0**
Dextrose	**1.0**
Ferric citrate	**0.3**
Sodium thiosulphate	**0.3**
Phenol red	**q.s.**
Agar No. 3 (Oxoid L13)	**12.0**

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 65 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Mix well and distribute. Sterilize by autoclaving at 121° C for 15 minutes. Allow the medium to set in sloped form with a butt about 1 in. long.

Tablets: Add 1 tablet to 10 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes. Allow slopes to set with a generous butt.

DESCRIPTION

A composite medium for the differentiation of Enterobacteriaceae according to their ability to ferment lactose, sucrose and dextrose, and to produce hydrogen sulphide.

Not only does this medium perform most of the functions of Kligler Iron Agar but, in addition, its sucrose content permits the recognition and exclusion of *Paracolobactrum* and *Proteus* species. These organisms attack lactose slowly or not at all during the incubation period, but they attack sucrose readily. Because some *Proteus* and other species may give similar reactions to salmonellae and shigellae, it is necessary to distinguish them by their ability to hydrolyze urea. For this reason Triple Sugar Iron Agar should be used in parallel with Urea Broth or Urea Agar.

This medium was formerly considered to be interchangeable with Kligler medium for the detection of hydrogen sulphide producing Enterobacteriaceae. It is now thought that Triple Sugar Iron Agar is not suitable for the detection of hydrogen sulphide production by sucrose fermenting organisms, such as some *Citrobacter* and *Proteus* species, in which the sucrose fermentation masks the hydrogen sulphide indicator in the medium (Bulmash & Fulton, 1964).

Triple Sugar Iron Agar is recommended for the presumptive identification of colonies or subcultures from plating media such as Salmonella Shigella Agar CM99, Bismuth Sulphite Agar (Single Pack) CM201, Brilliant Green Agar CM263, MacConkey Agar No. 3 CM115, or Desoxycholate Citrate Agar (Hynes) CM227.

TECHNIQUE

The American Techniques are described elsewhere (American Public Health Association, 1963, 1966; Edwards & Ewing, 1962; Society of American Bacteriologists, 1957) but the following is suggested as a simple alternative:

(1) Pick a single colony from the surface of a selective plating medium and smear a MacConkey Agar CM7 plate. Incubate for 18 hours at 37° C and inoculate two separate tubes of media from one single isolated colony:

(i) Triple Sugar Iron Agar—smear the slope and stab the butt.

(ii) Urea Broth Base CM71 (with added Urea Solution SR40).

(2) Incubate at 37° C.

(3) Examine the Urea Broth tube after 5 hours and again after 18 hours ncubation. Discard tubes showing a red or pink coloration, which is due to urea hydrolysis by *Proteus* or paracolon species.

(4) Where there is no urea hydrolysis, examine the Triple Sugar Iron Agar tubes after 18 hours and 48 hours. The following are typical reactions:

Organism	*Butt*	*Slope*	*H_2S*
Aerobacter aerogenes	AG	A	−
Aerobacter cloacae	AG	A	−
Escherichia coli	AG	A	−
Proteus vulgaris	AG	A	+
Proteus morganii	A or AG	NC or ALK	−
Shigella dysenteriae	A	NC or ALK	−
Shigella sonnei	A	NC or ALK	−
Salmonella typhosa	A	NC or ALK	+
Salmonella paratyphi	AG	NC or ALK	−
Salmonella schottmuelleri . .	AG	NC or ALK	+
Salmonella choleraesuis . . .	AG	NC or ALK	−
Salmonella enteritidis . . .	AG	NC or ALK	+
Salmonella typhimurium . . .	AG	NC or ALK	+

Nomenclature according to 'Bergey's Manual of Determinative Bacteriology' 7th ed.

AG = acid (yellow) and gas formation
A = acid (yellow)
NC = no change
ALK = alkaline (red)
+ = hydrogen sulphide (black) } see note on
− = no hydrogen sulphide (no black) } previous page.

The presumptive evidence so obtained may be confirmed serologically after subculturing the organism from the Triple Sugar Iron Agar slope in Nutrient Broth No. 2 CM67.

REFERENCES

American Public Health Association (1963) 'Diagnostic Procedures and Reagents' 4th ed., pp. 150 and 294–295. APHA Inc., New York.
American Public Health Association (1966) 'Recommended Methods for the Microbiological Examination of Foods' 2nd ed., APHA Inc., New York, pp. 158 and 185.
Bulmash, J. M. and Fulton, M. (1966) 'Discrepant Tests for Hydrogen Sulphide' *J. Bact.* **88**(6), 1813.
Edwards, P. R. and Ewing, W. H. (1962) 'Identification of *Enterobacteriaceae*' Burgess Publishing Co., Minneapolis.
Society of American Bacteriologists (1957) 'Manual of Microbiological Methods' McGraw-Hill Book Co. Inc., London.

Tryptone
Tryptone T

See page 216.

Tryptone Glucose Extract Agar

Code CM127

FORMULA

'Lab-Lemco' Powder (Oxoid L29)	*grams per litre*	**3**
Tryptone (Oxoid L42)		**5**
Dextrose (Glucose)		**1**
Agar No. 3 (Oxoid L13)		**15**

pH 7.0 (approx.)

DIRECTIONS

Suspend 24 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. Mix well before pouring. When the dilution of the original specimen is greater than 1 in 10, add 10 ml of sterile 10% solution of Skim Milk powder (Oxoid L31) per litre.

DESCRIPTION

This medium, of American origin, is recommended for the plate count of milk and dairy products in 'Standard Methods for the Examination of Dairy Products' (1960). It may be also used for the detection of thermophilic bacteria in dairy products. The Standard Methods Committee of the American Public Health Association recommended that sterile milk should be added to this medium, only when the dilution of the original specimen was greater than 1 in 10. The addition of 10 ml of a sterile 10% solution of Skim Milk Powder (Oxoid L31) per litre is suitable for this purpose. It is essential that this medium is not overheated during sterilization.

See also Plate Count Agar CM325.

REFERENCE

American Public Health Association (1960) 'Standard Methods for the Examination of Dairy Products', 11th ed., APHA Inc., New York.

Tryptone Glucose Yeast Agar

See page 223.

Tryptone Soya Agar

Code—Powder CM131
Tablets CM132

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**15**
Soya Peptone (Oxoid L44)		**5**
Sodium chloride		**5**
Agar No. 3 (Oxoid L13)		**15**

pH 7.3 (approx.)

DIRECTIONS

Powder: Suspend 40 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A general purpose agar medium, containing two peptones, which will support the growth of a wide variety of organisms. It is suitable for the cultivation both of aerobes and anaerobes, the latter being grown either in deep cultures or by incubation under anaerobic conditions. The medium may also be used as a blood agar base—for this purpose 7% of sterile blood should be added to the sterile molten medium which has been cooled to approximately 45° C. Tryptone Soya Agar can also be used for the preparation of 'chocolate' agar.

Since Tryptone Soya Agar contains no added carbohydrate it may be used, with added blood, in the simultaneous determination of haemolysis and bacterial sensitivity to antimicrobial agents. See also Diagnostic Sensitivity Test Agar CM261.

Horse blood agar plates prepared with Oxoid Tryptone Soya Agar are used for the colicine typing of *Shigella sonnei* (Abbott & Graham, 1961; Barrow & Ellis, 1962; Cooke & Daines, 1964; Gillies, 1964).

The Oxoid medium has also been used as a replacement for yeastrel-milk agar plates in the Lisboa test (Mitchell, 1964) and for bacterial counts on eviscerated poultry (Barnes & Shrimpton, 1958).

REFERENCES

Abbott, J. D. and Graham, J. M. (1961) 'Colicine Typing of *Shigella sonnei*' *Mon. Bull Min. Hlth Pub. Hlth Lab. Serv.* **20,** 51–58.

Barnes, Ella M. and Shrimpton, D. H. (1958) 'The Effect of the Tetracycline Compounds on the Storage Life and Microbiology of Chilled Eviscerated Poultry' *J. appl. Bact.* **21**(2), 313–329.

Barrow, G. I. and Ellis, C. (1962) 'Colicine Typing of *Shigella sonnei* by Replicate Multiple-Slide Inoculation of Indicator Organisms' *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv.* **21,** 141–147.

Cooke, G. T. and Daines, C. F. (1964) 'Colicine Types of *Shigella sonnei* in West Surrey' *Mon. Bull. Min. Hlth Pub. Hlth Lab. Serv.* **23,** 81–85.

Gillies, R. R. (1964) 'Colicine Production as an Epidemiological Marker of *Shigella sonnei*' *J. Hyg.* Camb. **62**(1), 1–9.

Mitchell, T. G. (1964) 'Some Factors Affecting the Lisboa Surface Film Test for Detergent-Sterilizers' *J. appl. Bact.* **27**(1), 45–52.

Tryptone Soya Broth

Soybean Casein Digest Medium U.S.P.

Code—Powder CM129
Tablets CM130

FORMULA

Pancreatic Digest of Casein (Oxoid L42) *grams per litre*	**17.0**
Papaic Digest of Soybean Meal (Oxoid L44)	**3.0**
Sodium chloride	**5.0**
Dibasic potassium phosphate	**2.5**
Dextrose	**2.5**

pH 7.3 (approx.)

DIRECTIONS

Powder: Add 30 g to 1 litre of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.
Tablets: Add 1 tablet to 10 ml of distilled water and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A highly nutritious versatile medium which is recommended for general laboratory use. Due to the inclusion of both Tryptone and Soya Peptone, the medium will support a luxuriant growth of many fastidious organisms without the addition of serum, etc.

TECHNIQUE

Aerobic Cultivation. Tryptone Soya Broth may be used for the cultivation of aerobes and facultative anaerobes, including some fungi. Cultures should be examined at frequent intervals, as maximum growth is reached earlier than with less nutritious media—and the phase of decline consequently begins sooner.

Anaerobic Cultivation. The addition of a small amount of agar (e.g. up to 1 Agar Tablet CM49 to every 100 ml of reconstituted Tryptone Soya Broth, prior to sterilization) renders the broth suitable for the cultivation of obligatory anaerobes, such as *Clostridium* species. For this purpose, the broth (with added agar) should be used soon after sterilization, or, heated and cooled just before inoculation.

Blood Culture. The superior growth-promoting properties of Tryptone Soya Broth make it especially useful for the isolation of organisms from blood or other body fluids. Anticoagulants such as 'Liquoid'* (sodium polyanethyl sulphonate) or sodium citrate may be added to the broth prior to sterilization. Five to 10 ml of blood may be added to 50 ml of medium.

Tryptone Soya Broth is especially suitable for the tube dilution method of antibiotic sensitivity testing.

Oxoid laboratory tests have shown that Tryptone Soya Broth has a greater ability to resuscitate heated spores of *Bacillus stearothermophilus* than Dextrose Tryptone Broth. Tryptone Soya Broth is recommended in the U.S.P. XVIII (Soybean Casein Digest Medium p.852) for the recovery of organisms after sterilization processes. The change of medium also overcomes the objections of workers to the inclusion of brom-cresol purple in the recovery broth (Cook & Brown, 1960). A positive result is revealed after 24–48 hours incubation at 55° C, by a heavy growth of organisms causing turbidity in the broth.

Oxoid Spore Strips (BR23) carry directions recommending Tryptone Soya Broth as the recovery medium.

REFERENCE

Cook, A. M. and Brown, M. P. W. (1960) 'Preliminary Studies of Heat-resistant Bacteria, Spores on Paper Carriers' *J. Pharm. Pharmacol.* **12,** Suppl. 16T 118T.

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* Obtainable in 1 gram and 10 gram bottles from Roche Products Ltd., Broadwater Road, Welwyn Garden City, Herts.

Tryptone Water

Code—Powder CM87
Tablets CM88

FORMULA

Tryptone (Oxoid L42)	*grams per litre*	**10**
Sodium chloride		**5**

pH 7.5 (approx)

DIRECTIONS

Powder: Add 15 g to 1 litre of distilled water. Mix well, distribute into final containers and sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

Tryptone Water has been specially evolved as a substrate for the production of indole. Due to its high content of tryptophan, it is more reliable than peptone water for this purpose. The ability of certain organisms to break down the amino-acid tryptophan with formation of indole is an important property which is used for the classification and identification of bacteria.

TECHNIQUE

Optimum time and temperature of incubation will vary considerably according to the organisms being investigated, and there are also a considerable number of tests for the detection of indole:

1. An adaptation of an American method (American Public Health Association, 1955), used for the differentiation of coliform organisms, is as follows:

(i) Test Reagent (Kovacs):

paradimethylaminobenzaldehyde	5 grams
amyl alcohol	75 ml
concentrated hydrochloric acid	25 ml

(ii) Inoculate tubes of Tryptone Water, and incubate for 24 hours at 35° C, or for 48 hours at 37° C.

(iii) Add 0.2 ml of Test Reagent and shake. Allow to stand for 10 minutes and observe the result.

A dark red colour in the amyl alcohol surface layer constitutes a positive indole test; no change in the original colour of the reagent constitutes a negative test.

2. Ehrlich reagent, described by Mackie & McCartney (1956), may be used as follows:

(i) Test Reagent (Ehrlich rosindole reagent):

paradimethylaminobenzaldehyde	4 grams
absolute alcohol	380 ml
concentrated hydrochloric acid	80 ml

(ii) Inoculate tubes of Tryptone Water, and incubate for 2 days at 37° C.

(iii) Withdraw a small portion of the culture and add an equal volume of the reagent. Shake the mixture and allow to stand for a few minutes, a rose colour indicates the presence of indole. If indole is present, this colour change may be accelerated by the addition of saturated potassium persulphate solution.

As an alternative, extract the indole by shaking the cultures with 1 ml of ether, allow the mixture to stand for a few minutes and then add 0.5 ml of Ehrlich reagent.

REFERENCES

American Public Health Association, American Water Works Association, Federation of Sewage and Industrial Wastes Association (1955) 'Standard Methods for the Examination of Water, Sewage, and Industrial Wastes', 10th ed., APHA Inc., New York, pp. 391–392.

Mackie, T. J. and McCartney, J. E. (1956) 'Handbook of Practical Bacteriology', 9th ed., Livingstone Ltd., Edinburgh and London, pp. 427–428.

Tryptose

See page 217.

Tryptose Blood Agar Base

Code CM233

FORMULA

Tryptose (Oxoid L47)	*grams per litre*	**10**
'Lab-Lemco' Powder (Oxoid L29)		**3**
Sodium chloride		**5**
Agar No. 3 (Oxoid L13)		**12**

pH 7.2 (approx.)

DIRECTIONS

Suspend 30 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

For blood agar, cool the basal medium to 45–50° C and add 7% of sterile blood. Mix thoroughly, taking care to avoid incorporation of air bubbles, and dispense into Petri dishes or other containers.

DESCRIPTION

A highly nutritious medium specially developed for the preparation of a blood agar which will support the growth of many fastidious organisms.

Tryptose Phosphate Broth

Code CM283

FORMULA

Tryptose (Oxoid L47)	*grams per litre*	**20.0**
Dextrose		**2.0**
Sodium chloride		**5.0**
Disodium hydrogen phosphate		**2.5**

pH 7.3 (approx.)

DIRECTIONS

Add 29.5 g to 1 litre of warm distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121° C for 15 minutes.

For the cultivation of anaerobes, if the reconstituted medium has been stored prior to use, remove dissolved oxygen by placing the tubes in a boiling water bath for 15 minutes and cool without agitation before inoculation.

DESCRIPTION

A buffered dextrose broth for use as an adjuvant to tissue culture media and for the cultivation of fastidious bacteria. Ginsberg *et al.* (1955) maintained tissue cultures of HeLa cells for at least 10 days in a mixture of Tryptose Phosphate Broth 15–25%, Scheren maintenance solution 67.5–77.5% and chicken serum 7.5%. The cells increased 3–5 fold in number during this period, smaller quantities of ARD, AD and type 1 poliomyelitis virus could be detected and more ARD virus could be propagated in HeLa cells in the Tryptose Phosphate Broth supplemented medium. Tryptose Phosphate Broth is recommended for the cultivation of streptococci, pneumococci, meningococci and other fastidious organisms. The medium is also suitable for the tube method of testing antibiotic sensitivity (Waisbren *et al.*, 1951).

Tryptose Phosphate Broth with added agar and sodium azide is recommended for the isolation of pathogenic streptococci from cheese and other dairy products (American Public Health Association, 1953; Newman, 1950).

Tryptose Phosphate Broth with added agar is also recommended by the American Public Health Association (1950) for the examination of throat swabs and blood for *Streptococcus pneumoniae*, and as a growth medium for pneumococci prior to the bile solubility test.

Tryptose Phosphate Agar Broth may also be employed for the emulsification of cheese prior to the plate isolation method for *Brucella* species (American Public Health Association, 1953).

The small proportion of agar (0.1–0.2%) necessary for the above methods may be most conveniently added to the Oxoid broth before sterilization, in the form of Oxoid Agar Tablets CM49 (1 tablet per 100 ml).

TECHNIQUE

Examination of Swabs for Pneumococci (APHA, 1963).

Prepare Tryptose Phosphate Broth in the usual manner but add a small amount of agar by dissolving 10 Agar Tablets CM49 per litre of broth before autoclaving.

Place a pharyngeal or sputum swab in 3 ml of Tryptose Phosphate (agar) Broth and incubate for 2 hours at 37° C. If sufficient pneumococci are present, type directly or preferably employ the culture for mouse inoculation.

Examination of Cheese for Lancefield Group A Streptococci (APHA, 1953). Heat 25 ml of Tryptose Phosphate (agar) Broth (prepared as above) to 45° C. Immediately emulsify 5 grams of cheese in the broth, using a heavy sterile glass rod. Plate suitable dilutions of the emulsion on tryptose agar plates containing 1.5% and 2.5% of sodium azide.

Alternatively, add sterile aqueous sodium azide to the emulsion to give a final concentration of 2.5%, incubate for 12–14 hours at 35° C, shake, plate on Tryptose Agar plates containing 1.5% and 2.5% of sodium azide.

The sodium azide suppresses the growth of most bacteria except streptococci and *Lactobacillus* type organisms.

Blood Culture

Add up to 10 ml of blood to 150 ml of Tryptose Phosphate Broth in a 300 ml Erlenmeyer flask. Incubate and subculture on to other media in the usual manner, according to the exact purpose of the investigation.

REFERENCES

American Public Health Association (1963) 'Diagnostic Procedures and Reagents' 4th ed., APHA Inc., New York, p. 141.

American Public Health Association (1953) 'Standard Methods for the Examination of Dairy Products' 10th ed., APHA Inc., New York, pp. 179, 180, 181.

Ginsberg, H. S. *et al.* (1955) 'Tryptose Phosphate Broth as Supplementary Factor for Maintenance of HeLa Cell Tissue Cultures' *Proc. Soc. Exper. Biol. Med.* **89(1)**, 66–71.

Newman, R. W. (1950) 'Laboratory Detection of Food Poisoning Attributable to Dairy Products' *J. Milk Food Tech.* **13**, 226–233.

Waisbren, B. A. *et al.* (1951) 'The Tube Dilution Method of Determining Bacterial Sensitivity to Antibiotics' *Am. J. Clin. Path.* **21**, 884–891.

Urea 40%

Code SR20

For inclusion in Kohn Two-Tube Medium No. 1 CM179, Urea Agar Base CM53, and Urea Broth Base CM71. This filter sterilized solution is particularly useful because urea solution may not be sterilized by the application of heat. Available in ½ ml and 5 ml ampoules, in boxes of ten.

Urea Agar Base

Code—Powder CM53
Tablets CM54

FORMULA

Peptone (Oxoid L37)	*grams per litre*	**1.0**
Dextrose		**1.0**
Sodium chloride		**5.0**
Disodium phosphate		**1.2**
Potassium dihydrogen phosphate		**0.8**
Phenol Red		**0.012**
Agar No. 3 (Oxoid L13)		**15.0**

pH 6.8 (approx.)

DIRECTIONS

Powder: Suspend 2.4 g in 95 ml of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 115° C for 20 minutes. Cool to 50° C and aseptically introduce 5 ml of sterile 40% Urea Solution (Oxoid SR20). Mix well, distribute 10 ml amounts into sterile containers and allow to set in the slope position.

Tablets: Add 1 tablet to 9.5 ml of distilled water. Sterilize by autoclaving at 115° C for 20 minutes. Cool to 55° C, aseptically add 0.5 ml of sterile 40% Urea Solution (SR20). Mix well, slope.

DESCRIPTION

Urea Agar Base is recommended for the preparation of Christensen medium (Christensen, 1946) for the detection of urea splitting organisms such as *Proteus vulgaris*. The urea medium may be used for the detection of ureal hydrolysis by other micro-organisms, including certain micrococci and paracolon organisms.

TECHNIQUE

Heavily inoculate the surface of a Urea Agar slope with a pure culture of the organism to be tested. The reaction is usually complete after 3 to 5 hours at 37° C: urease producing organisms hydrolyse the urea to form ammonia, and the medium changes to purple-red.

40% Urea Solution is supplied, as a sterile solution in ampoules, for the convenient preparation of this medium.

REFERENCE

Christensen, W. B. (1946) 'Urea Decomposition as a means of Differentiating *Proteus* and paracolon Cultures from each other and from *Salmonella* and *Shigella* Types' *J. Bact.* **52**, 461–466.

Urea Broth Base

Code CM71

FORMULA

	grams per litre
Peptone (Oxoid L37)	1.0
Dextrose	1.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Sodium chloride	5.0
Phenol red	0.004

pH 6.8 (approx.)

DIRECTIONS

Add 0.9 g to 95 ml of distilled water. Sterilize by autoclaving at 115° C for 20 minutes. Cool to 55° C and aseptically introduce 5 ml of sterile 40% Urea Solution (Oxoid SR20). Mix well and distribute 10 ml amounts into sterile containers.

DESCRIPTION

This is a liquid modification of Christensen medium (Maslen, 1952). The modification is suitable for the differentiation of urease producing organisms from members of the *Salmonella* and *Shigella* groups, during the routine examination of rectal swabs and faeces. Maslen noted that in the routine examination of faeces for *Salmonella* and *Shigella* organisms many non-lactose fermenting colonies isolated were later found to belong to the *Proteus* and paracolon groups. He evolved this medium as a means whereby the latter organisms could be rapidly detected and eliminated—thus saving a considerable amount of time and media. Maslen claimed that the advantages of the fluid medium were:

(i) A Pasteur pipette could be used to inoculate other diagnostic media.

(ii) Rapid growth ensued and it was possible to discern a clear-cut positive reaction within two to five hours at 37° C.

(iii) It was easier to detect any contamination during storage.

TECHNIQUE

For the examination of faeces, specimens are cultured in selective and enrichment media in the usual manner. Discrete colonies are then picked off the surface of the solid selective media.

Inoculate tubes of Urea Broth with single colonies of non-lactose fermenting organisms and incubate for 5 to 6 hours at 37° C. (Maslen states that the cultures should be incubated in a water bath in order to obtain the highest proportion of positive reactions within 5 hours.) Regard all organisms producing a pink coloration in the medium (i.e. due to the alkalinity caused by urea hydrolysis) as not belonging to the *Salmonella* or *Shigella* groups, and discard.

Inoculate all cultures showing no colour change (i.e. no urea hydrolysis) into 'sugar' peptone waters (Phenol Red Peptone Water CM63 or Andrade Peptone Water CM61) plus the appropriate carbohydrate (see SR range) and on to a Blood Agar Base CM55 slope.

Incubate the new cultures, together with the Urea Broth, until the next morning. No further examination is necessary if the urea tube now shows an alkaline reaction (pink colour), otherwise continue the diagnostic tests—including slide agglutinations from the Blood Agar Base culture, if necessary.

REFERENCE

Maslen, L. G. C. (1952) 'Routine Use of Liquid Urea Medium for Identifying *Salmonella* and *Shigella* Organisms' *Brit. Med. J.* **2**, 545–546.

V Factor Discs

See page 288.

Violet Peptone Bile Lactose Broth

Code CM223

FORMULA

	grams per litre
Peptone (Oxoid L37)	10.0
Lactose	10.0
Bile Salts (Oxoid L55)	5.0
Gentian violet	0.04

pH 7.6 (approx.)

DIRECTIONS

Add 25 g to 1 litre of distilled water. Mix well and distribute into containers fitted with Durham's tubes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

A selective liquid medium, recommended by the Methoden-kommission für Milchwirtschaft for the determination of the *coli-aerogenes* group in milk and milk products. Gas production is detected with the aid of a small inverted tube (Coli-Glöckchen). The selective action of this medium is due to the incorporation of Bile Salts and gentian violet.

REFERENCES

Methodenbuch Band VI. 'Untersuchung von Milch, Mildherzeugnissen und Molkereihilfsstoffen'. Herausgegeben von Verband Deutscher Landwirtschaftlicher Untersuchungs-und-Forschungsanstalten, Neumann Verlag, Radebeul und Berlin 1950, Seite 116.
Demeter, 'Bakteriologische Untersuchungsmethoden der Milchwirtschaft', Ulmer Verlag, Stuttgart. 1952, Seite 58–60.
Kessler and Swenarton (1927) *J. Bact.* **14**, 47–53.

Violet Red Bile Agar

Code—Powder CM107
Tablets CM108

FORMULA

Yeast Extract Powder (Oxoid L21) *grams per litre*	3.0
Peptone (Oxoid L37)	7.0
Sodium chloride	5.0
Bile Salts No. 3 (Oxoid L56)	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar No. 3 (Oxoid L13)	12.0

pH 7.4 (approx.)

DIRECTIONS

Powder: Suspend 38.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. No further sterilization is necessary or desirable. Mix well before pouring.

Tablets: Add 1 tablet to 5 ml distilled water, soak for 5 minutes, bring to the boil to dissolve the medium completely. No further sterilization is necessary.

DESCRIPTION

Violet Red Bile Agar is a selective medium for the detection and enumeration of coliform organisms. The medium has been used for the determination of the *coli-aerogenes* content of water, milk and other dairy products, dairy equipment, and food products, etc.

For the presumptive test for coliform organisms, in dairy products (using solid media), methods using Violet Red Bile Agar are recommended by Davis (1951) and the American Public Health Association (1967). Druce *et al.* (1957) found that Violet Red Bile Agar was as good an indicator of *coli-aerogenes* bacteria in milk as MacConkey broth, and that the Oxoid medium was suitable for determining the *coli-aerogenes* content of milk.

TECHNIQUE

Druce *et al.* recommended the following procedures:

For the routine determination of the *coli-aerogenes* content of raw milk, prepare pour-plates containing 1.0, 0.1 and 0.01 ml of the sample in Violet Red Bile Agar, and incubate for 20–24 hours at 37° C.

For *coli-aerogenes* counts of pasteurized milk, employ 4 pour-plates of Violet Red Bile Agar. Divide 10 ml of the sample among three of the plates, and add 1 ml of the sample to the remaining plate. Incubate for 20 to 24 hours at 30° C. Similarly the examination of rinses and swabs from dairy equipment and apparatus, should include the spreading of 10 ml of solution on each of 3 plates and of 1 ml on a single plate. Coliform organisms form dark red colonies which are 1 to 2 mm in diameter, usually surrounded by a reddish zone. Occasional *coli-aerogenes* may be considerably smaller (less than 0.5 mm in diameter).

This medium may also be employed for roll tube work, but the volume of inoculum should be compensated for by a corresponding reduction in the water for reconstitution.

REFERENCES

American Public Health Association (1967) 'Standard Methods for the Examination of Dairy Products', 12th ed., APHA Inc., New York, pp. 56, 58 and 241.
Davis, J. G. (1951) 'Milk Testing', Dairy Industries Ltd., London, pp. 131–132.
Druce, R. G., Bebbington, N. N., Elson, K., Harcombe, J. M. and Thomas, S. B. (1957) 'The Determination of the *coli-aerogenes* Content of Milk and Dairy Equipment by Plating on Violet Red Bile Agar Incubated at 30°' *J. appl. Bact.* **20**(1), 1–10.

Violet Red Bile Dextrose Agar

Code CM323

FORMULA

Yeast Extract Powder (Oxoid L21) *grams per litre*	3.0
Bacteriological Peptone (Oxoid L34)	7.0
Sodium chloride	5.0
Bile Salts No. 3 (Oxoid L56)	1.5
Lactose	10.0
Dextrose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar No. 3 (Oxoid L13)	12.0

pH 7.4 (approx.)

DIRECTIONS

Suspend 48.5 grams in 1 litre of distilled water. Boil to dissolve the medium completely. **No further sterilization is necessary or desirable.** Mix well and dispense into tubes or petri dishes.

DESCRIPTION

Violet Red Bile Dextrose Agar (CM323) was formulated by Mossel, Mengerink and Scholts (1962) for the development of virtually all of the Enterobacteriaceae in food, animal feedstuffs, pharmaceutical products, cosmetics, etc.

Tests with bacteria not belonging to the Enterobacteriaceae showed that the vast majority of these organisms did not grow. Only obligate aerobic, Gram-negative, rod-shaped bacteria (e.g. *Achromobacter anitratus, Aeromonas spp., Pseudomonas spp.*) will grow, although these are generally inhibited in pour plates.

Violet Red Bile Dextrose Agar (CM323) is of value in the detection of anaerobic coliform organisms which would be missed by the traditional gas-production techniques (Mossel & Krugers Dagneaux, 1959).

The use of E.E. Broth (CM317) in combination with Violet Red Bile Dextrose Agar has been shown by Mossel, Visser and Cornelissen (1963) to overcome problems associated with the isolation of Enterobacteriaceae from food samples.

Mossel & Ratto (1970) demonstrated that one or two hours resuscitation in Oxoid Tryptone Soya Broth (CM129) prior to enrichment in E.E. Broth (CM317) gave significantly more productive results in dried foods, feeds and drugs.

Using this technique 1 g samples can be examined by resuscitation of 1:10 dilutions in Tryptone Soya Broth (CM129). The broth dilutions should be placed into sterile flasks so that the fluid layers are not deeper than one centimetre. Shake to disperse, alternatively in a clockwise and anti-clockwise direction for 30 seconds on three successive occasions. Incubate at room temperature for 8 hours, repeating the shaking procedure after the first 3 hours incubation. After the period of resuscitation add the product under examination to 10-fold volumes of E.E. Broth and incubate at 31 ± 1° C for 18 to 24 hours.

For larger samples it is desirable to add the resuscitation medium, containing the product under examination to equal volumes of double-strength E.E. Broth (CM317).

The enrichment cultures are stabbed into freshly steamed deep tubes of Violet Red Bile Dextrose Agar, cooled to 30° C. After 18 to 24 hours incubation at 30° C the tubes are inspected for the typical growth pattern of Enterobacteriaceae, i.e. development of an inoculation line reaching to the bottom of the tube, surrounded by a coaxial cylinder of purple precipitate, with or without gas production.

Pseudomonadaceae are not able to attack dextrose under the anaerobic conditions in a stab culture.

Further differentiation of cultures in the lowest part of the agar column can be made by streaking on the dry surface of Violet Red Bile Dextrose Agar (CM323) plates. Every colony which shows a purple halo on a purple background can be considered as presumptive evidence of the presence of Enterobacteriaceae, Oxidase-negative colonies, tested by applying Kovacs' oxidase test, indicate with certainty the presence of Enterobacteriaceae in the product under examination.

Aeromonas spp. will give a positive oxidase reaction and may be excluded from the Enterobacteriaceae with little difficulty.

Further identification reactions on the Enterobacteriaceae can be carried out as required.

REFERENCES

Mossel, D. A. A., Mengerink, W. H. J. and Scholts, H. H. (1962) 'Use of a modified MacConkey Agar medium for the selective growth and enumeration of Enterobacteriaceae' *J. Bact.* **84,** 381.

Mossel, D. A. A., Visser, M. and Cornelissen, A. M. R. (1963) 'The examination of foods for Enterobacteriaceae using a test of the type generally adopted for the detection of salmonellae' *J. appl. Bact.* **26,** 444–452.

Mossel, D. A. A. and Krugers Dagneaux, E. L. (1959) 'Bacteriological requirement for and bacteriological analysis of pre-cooked ("instant") cereals and similar foods' *Antonie van Leeuwenhoek* **25,** 230–236.

Mossel, D. A. A. and Ratto, M. A. (1970) 'Rapid detection of sub-lethally impaired cells of Enterobacteriaceae in dried foods' *Appl. Microbiol.* **20,** 273–275.

Wilson and Blair Medium

See page 61.

W. L. Nutrient Medium

Code CM309

FORMULA

Yeast Extract Powder (Oxoid L21) *grams per litre*	**4.0**
Tryptone (Oxoid L42)	**5.0**
Dextrose	**50.0**
Potassium dihydrogen phosphate	**0.55**
Potassium chloride	**0.425**
Calcium chloride	**0.125**
Magnesium sulphate	**0.125**
Ferric chloride	**0.0025**
Manganese sulphate	**0.0025**
Brom-cresol green	**0.022**
Agar No. 3 (Oxoid L13$_2$	**15.0**

pH 5.5 (approx.)

DIRECTIONS

Suspend 75 grams in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes. If required the pH may be adjusted to 6.5 by the addition of 1% sodium bicarbonate solution.

DESCRIPTION

'Oxoid' W.L. Nutrient Medium, based on that of Green & Gray (1950) is recommended for the determination of the microbiological flora in brewing and fermentation processes. The medium is suitable for the differentiation of 'wild' yeasts from brewing yeasts (Hall, 1971).

Reliable counts for brewer's yeast are obtained with the medium at pH 5.5. Adjustment to pH 6.5 facilitates the counting of baker's and distiller's yeasts.

With the addition of 'Actidione' 0.004 grams per litre, to suppress yeast growth, the medium becomes selective for bacterial contaminants of yeast cultures.

REFERENCES

Green, S. R. and Gray, P. P. (1950) 'Differential Procedure Applicable to Bacteriological Investigation in Brewing' *Wallerstein Lab. Comm.* **13**, 357.

Hall, Jean F. (1971) 'Detection of Wild Yeasts in the Brewery' *J. Inst. Brewing* **77**, 513–516.

Wort Agar

Code—Powder CM247
Tablets CM248

FORMULA

Malt Extract (Oxoid L39) *grams per litre*	**15.0**
Peptone (Oxoid L37)	**0.78**
Maltose	**12.75**
Dextrin	**2.75**
Glycerol	**2.35**
Dipotassium phosphate	**1.0**
Ammonium chloride	**1.0**
Agar No. 1 (Oxoid L11)	**15.0**

pH 4.8 (approx.)

DIRECTIONS

Powder: Suspend 50 g in 1 litre of distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

PROLONGED OR EXCESSIVE HEATING WILL DIMINISH THE GEL STRENGTH OF THE AGAR.

DESCRIPTION

Wort Agar is a general purpose mycological medium, equivalent to the medium described by Parfitt (1933) and especially suitable for the cultivation and enumeration of yeasts. The medium which duplicates the composition of natural wort, is of an acidity which is optimal for many yeasts but inhibitory to most bacteria. Parfitt investigated the relative merits of wort agar and other media for the count of yeasts and moulds in butter, and recommended the use of dehydrated whey, malt or wort agar for the purpose. Scarr (1959) employed a modified Wort Agar ('osmophilic agar') for the examination of sugar products for osmophilic yeasts. Scarr's technique is also used for the determination of osmophilic yeasts occurring in materials used in the manufacture of soft drinks.

TECHNIQUE

For the microbiological examination of butter, make suitable dilutions in quarter-strength Ringer solution (prepared with Ringer Solution Tablets BR52). Transfer 1 ml of each dilution to a separate Petri dish; add 15 ml of melted Wort Agar, cooled to 45° to 48° C; mix by rotary movements in a horizontal plane; allow the poured-plates to set (protected from the light) at room temperature for 30–60 minutes. Incubate in an inverted position, e.g. for 5 days at 25° C, and count the number of yeasts and mould colonies which develop.

For the examination of sugar products for osmophilic yeasts, dissolve dehydrated wort agar in a syrup containing 35 parts w/w of sucrose and 10 parts w/w of glucose, and autoclave for 20 minutes at 110° C. Inoculate and mix as above. Scarr recommends incubation at 27° C for 3–4 days for *Schizosaccharomyces* species and for 5 days for less common osmophils. Colonies on the medium are well defined and normally opaque.

REFERENCES

Parfitt, E. H. (1933) 'The Influence of Media upon the Yeast and Mold Count of Butter' *J. Dairy Sci.* **16**, 141–147.

Scarr, M. Pamela (1959) 'Selective Media used in the Microbiological Examination of Sugar Products' *J. Sci. Food Agric.* **10**(12), 678–681.

XLD Medium

Code CM469

FORMULA

Yeast Extract Powder (Oxoid L21) *grams per litre*	**3.0**
L-Lysine HCl	**5.0**
Xylose	**3.75**
Lactose	**7.5**
Sucrose	**7.5**
Sodium desoxycholate	**1.0**
Sodium chloride	**5.0**
Sodium thiosulphate	**6.8**
Ferric ammonium citrate	**0.8**
Phenol red	**0.08**
Agar No. 1 (Oxoid L11)	**12.5**

pH 7.4 (approx.)

DIRECTIONS

Suspend 55 g in 1 litre of distilled water. Heat with frequent agitation until the medium boils. DO NOT OVERHEAT. Transfer immediately to a water bath at 50° C. Pour into plates as soon as the medium has cooled.

It is important to avoid preparing large volumes which will cause prolonged heating.

DESCRIPTION

Xylose Lysine Desoxycholate Medium was originally formulated by Taylor (1965) for the isolation and identification of shigellae from stool specimens. It has since been found to be a satisfactory medium for the isolation and presumptive identification of both salmonella and shigella (McCarthy 1966).

It relies on xylose fermentation, lysine decarboxylation and production of hydrogen sulphide for the primary differentiation of shigellae and salmonellae from non-pathogenic bacteria.

Rapid xylose fermentation is almost universal amongst enteric bacteria, except for members of the Shigella, Providencia and Edwardsiella groups (Taylor, 1965). Xylose is thus included in the medium so that shigella species may be identified by a negative reaction.

Salmonella species are differentiated from non-pathogenic xylose fermenters by the incorporation of lysine in the medium. Salmonellae exhaust the xylose and decarboxylate the lysine, thus altering the pH to alkaline and mimicking the Shigella reaction. However, the presence of Salmonella, Arizona and Edwardsiella species is differentiated from that of Shigellae by a hydrogen sulphide indicator.

The high acid level produced by fermentation of lactose and sucrose, prevents lysine-positive coliforms from reverting the pH to an alkaline value, and non-pathogenic hydrogen sulphide producers do not decarboxylate lysine. The acid level also prevents blackening by these micro-organisms until after the 18 to 24 hour examination for pathogens.

Sodium desoxycholate is incorporated as an inhibitor in the medium. The concentration used allows for the inhibition of coliform without decreasing the ability to support shigellae and salmonellae.

The recovery of Shigella species has previously been neglected despite the high incidence of shigellosis. This has been due to inadequate isolation media (Isenberg, Kominos & Shiegel, 1969). The sensitivity and selectivity of XLD Medium exceeds that of the traditional plating media, e.g. Eosin Methylene Blue, Salmonella-Shigella, and Bismuth Sulphite agars, which tend to suppress the growth of shigellae. Many favourable comparisons between XLD Medium and these other media have been recorded in the literature (Taylor & Harris, 1965; McCarthy, 1966; Taylor & Harris, 1967; Taylor & Schelhart, 1967; Taylor & Schelhart, 1968; Rollender Beckford, Belsky & Kostroff, 1969; Taylor & Schelhart, 1969; Dunn & Martin, 1971).

Because recovery of salmonellae and shigellae is not obscured by profuse growth of other species (Isenberg *et al.* 1969), XLD Medium is ideal for the screening of samples containing mixed flora and suspected of harbouring enteric pathogens, e.g. medical specimens or food products. Chadwick, Delisle & Byer (1974) recommended the use of this medium as a diagnostic aid in the identification of Enterobacteriaceae.

TECHNIQUE

Faeces or rectal swabs may be plated directly (Weissman, Gangarosa, Schmerler, Marier & Lewis, 1975) or selective enrichment broths may be used prior to streaking out. Selenite Broth (Oxoid CM395) or Tetrathionate Broth (Oxoid CM29) may be used for Salmonella enrichment.

1. Inoculate the poured, dried plates with a loopful of inoculum either from a suitable enrichment broth, from stool samples or rectal swabs.

2. Incubate the plates at 37° C for 18 to 24 hours.

COLONIAL APPEARANCES

Organism	*Appearance*
Salmonella	Red colonies with black centres
Arizona	
Edwardsiella	
Shigella	Red colonies
Proteus providencia	
H_2S-negative Salmonella (e.g. *S. paratyphi A*)	
Escherichia	Yellow, opaque colonies
Aerobacter (Enterobacter)	
Klebsiella	
Citrobacter (Bethesda-Ballerup)	
Proteus (other species)	
Serratia	

N.B. False positive, red colonies may occur with some Proteus and Pseudomonas species.

REFERENCES

Chadwick, P., Delisle, G. H. and Byer, M. (1974) 'Biochemical identification of hospital enterobacteria by replica agar plating.' *Can. J. Microbiol.* **20**, 1653–1664.

Dunn, C. and Martin, W. J. (1971) 'Comparison of media for isolation of salmonellae and shigellae from faecal specimens' *Appl. Microbiol.* **22**, 17–22.

Isenberg, H. D., Kominos, S. and Siegal, M. (1969) 'Isolation of salmonellae and shigellae from an artifical mixture of faecal bacteria' *Appl. Microbiol.* **18** (4), 656–659.

McCarthy, M. D. (1966) 'The use of Xylose Lysine Desoxycholate Agar as a medium for the isolation of intestinal pathogens' *N.Z. J. med. Lab. Technol.* **20**, 127–131.

Rollender, M. A., Beckford, O., Belsky, R. D. and Kostroff, B. (1969) 'Comparison of Xylose Lysine Desoxycholate Agar and MacConkey Agar for the isolation of salmonellae and shigellae from clinical specimens' *Am. J. Clin. Path.* **51**(2), 284–286.

Taylor, W. I. (1965) 'Isolation of shigellae: 1. Xylose Lysine Agars: new media for isolation of enteric pathogens' *Am J. Clin. Path.* **44**(4), 471–475.

Taylor, W. I. and Harris, B. (1965) 'Isolation of shigellae: 2. Comparison of plating media and enrichment broths' *Am. J. Clin. Path.* **44**(4), 476–479.

Taylor, W. I. and Harris, B. (1967) 'Isolation of shigellae: 3. Comparison of new and traditional media with stool specimens' *Am. J. Clin. Path.* **48**(3), 350–355.

Taylor, W. I. and Schelhart, D. (1967) 'Isolation of shigellae: 4. Comparison of plating media with stools' *Am. J. Clin. Path.* **48**(3), 356–362.

Taylor, W. I. and Schelhart, D. (1968) 'Isolation of shigellae: 6. Performance of media with stool specimens' *Appl. Microbiol.* **16**(9), 1387–1392.

Taylor, W. I. and Schelhart, D. (1969) 'Isolation of shigellae: 7. Comparison of Gram-Negative Broth with Rappaprot's Enrichment Broth' *Appl. Microbiol.* **18**(3), 393–395.

Weissman, J. B., Gangarosa, E. J., Schmerler, A., Marier, R. L. and Lewis, J. N. (1975) 'Shigellosis in day care centres, *Lancet* **I** No. 7898, 88–90.

Xylose 10%

SR22

Filter sterilized solution, available in 5 ml ampoules, in boxes of ten.

X Factor Discs

Code DD3

X + V Factor Discs

Code DD5

V Factor Discs

Code DD4

DESCRIPTION

Paper discs impregnated with growth factors for the differentiation of the *Haemophilus* group of bacteria.

Haemophilus and *Bordetella* species may be identified according to whether basal media require the addition of 'X' and 'V' growth factors before growth will occur. X factor (haemin) and V factor (coenzyme I) may be directly incorporated into a basal medium or, more conveniently, are impregnated into paper discs or strips which are placed upon the surface of an inoculated basal medium. Colonies of those species which show growth only in the vicinity of a disc or strip impregnated with the particular growth factor are unable to synthesize it in optimal amounts (Everall, 1953).

TECHNIQUE

Evenly inoculate the surface of a Blood Agar Base plate (CM55 *without blood*) with the organism to be tested and aseptically apply the Diagnostic Discs in the following positions around the periphery of the plate (approximately 1 or 2 cm in from the edge of the medium):

X Factor Disc — 12 o'clock
V Factor Disc — 4 o'clock
X + V Factor Disc — 8 o'clock

Incubate overnight at 37° C or for 48 hours if necessary, and observe for growth or no growth in the neighbourhood of a disc. If the organism requires X factor alone, it will grow only in the vicinities of the X and X + V factor discs; if it requires V factor alone, it will grow only in the vicinities of the V and the X + V factor discs; if both X and V factors are required, it will grow only in the vicinity of the X + V factor disc.

Growth of Bacterial Species with and without X and V factors

	No growth factors	*With X factor*	*With V factor*	*With X + V factor*
H. influenzae	−	−	−	+
H. aegyptius . . . . . (Koch-Weeks bacillus)	−	−	−	+
H. para influenzae . . .	−	−	+	+
H. ducreyi	−	+	−	+
*B. pertussis**	+	+	+	+

+ Growth
− No growth

* Requires special media for initial isolation, e.g. Bordet-Gengou medium, but laboratory strains show adaptation.

N.B.—V and X + V FACTOR DISCS **MUST** BE STORED BELOW 0° C.

REFERENCE

Everall, P. H. (1953) 'A Plate Method for Demonstrating the Growth Factor Requirements of the Genus *Haemophilus*' *J. Med. Lab. Technol.* **11** (3), 181–184.

Yeast Extract Powder

Code L21

Although for many purposes the long established yeast extract paste, L20 was a very satisfactory and economical culture media ingredient, there is no doubt that the superior nutritional properties of yeast extract powder is in many cases advantageous. In addition, the lighter colour, neutral pH and ease of handling of the powder form offer many additional virtues. It should be used in culture media at a concentration of 0.3–0.5%.

Analysis:

Moisture	4.1%
Ash	11.5%
Salt	1.3%
Phosphate (as P_2O_5) . . .	3.2%
Total Nitrogen	10.5%
Amino Nitrogen	4.8%
Nicotinic Acid	1,250 ppm
Riboflavin	150 ppm

Yeast Extract Agar

Code—Powder CM19
Tablets CM20

FORMULA

Yeast Extract Powder (Oxoid L21)	*grams per litre*	**3.0**
Peptone (Oxoid L37)		**5.0**
Agar No. 3 (Oxoid L13)		**15.0**

pH 7.2 (approx.)

DIRECTIONS

Powder: Suspend 23 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121° C for 15 minutes.

Tablets: Add 1 tablet to 5 ml of distilled water and soak for 5 minutes. Sterilize by autoclaving at 121° C for 15 minutes.

DESCRIPTION

This medium is made to the formula described in 'The Bacteriological Examination of Water Supplies' (1970) and by Windle Taylor (1958) for the plate count of micro-organisms in water.

TECHNIQUE

(i) Prepare appropriate decimal dilutions of the water sample (with Ringer Solution CM52) and pipette 1 ml portions of the water and each dilution into separate sterile Petri dishes.

(ii) Add 20 ml of Yeast Extract Agar (previously melted and cooled to 45–50° C) to each plate. Mix the contents by a combination of rapid to-and-fro shaking and circular movements lasting over a period of 5–10 seconds.

(iii) Allow to solidify, and incubate duplicate sets of plates for 24 hours at 37° C and 3 days at 20–22° C respectively.

(iv) Select plates containing 30–300 colonies for counting. No count should be made on a plate containing less than 30 colonies unless the plates from the undiluted water contain less than this number.

REFERENCES

Dept. of Health & Social Security (1970) Report No. 71: 'The Bacteriological Examination of Water Supplies', HMSO, London, pp. 7, 34–35, 45.

Windle Taylor, E. (1958) 'The Examination of Waters and Water Supplies', 7th ed., Churchill Ltd., London, pp. 394–398 and 778.

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